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REVIEW

Next-generation clinically relevant antibody detection: Unlocking electrochemical biosensors for critical disease management

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Immunoassay method

Abstract Autoimmune diseases, cancers, and viral infections pose significant global health threats, characterized by chronic pathology, unregulated cellular proliferation, and rapid transmission, respectively, requiring urgent early warning and treatment strategies. Antibodies, primarily classified into autoantibodies and therapeutic antibodies based on their clinical roles, provide essential information and show considerable value in the precise diagnosis and treatment of these serious diseases. Among the technologies utilized in bioanalysis, electrochemical biosensors, with their unique advantages of rapid response, high sensitivity, miniaturization, cost-effectiveness and user-friendly operation, have been developed as a trending technology for precise diagnostic and therapeutic drug monitoring. This review systematically summarizes the relationships and roles of clinically relevant antibodies in autoimmune diseases, cancers, and viral infections, while detailing the composition, strategies, development, and application trends of relevant electrochemical biosensors. Furthermore, it highlights the remaining challenges and opportunities for the advancement and prospects of electrochemical sensors in the context of clinically relevant antibodies.

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1. Introduction

Critical illnesses, including autoimmune diseases, cancers, and infectious diseases, have emerged as serious threats to human health[1]. These diseases often progress to immune system dysfunction, including hyperactivation or turning off the host response. Among them, more than 80 types of autoimmune diseases can involve essentially any organ system of the human body and affect individuals of any age[2,3]. Cancer is still the second leading cause of death globally, accounting for nearly 10 million deaths every year. After the critical illness caused by coronavirus disease 2019 (COVID-19), viral infections remain the leading cause of pandemics, representing a grave threat to public health[4,5]. Therefore, the prevention, diagnosis, and treatment of these critical illnesses are crucial. Recently, antibodies, including autoantibodies (AAbs) and therapeutic antibodies, have been widely used in personalized diagnosis and therapies across different diseases. For instance, AAbs, Y-shaped proteins generated mainly by differentiated B cells, might be detected before disease onset and thus possess remarkable specificity to serve as biomarkers enabling the prevention, diagnosis, and therapeutic tracking of diseases[6]. Moreover, due to the advancements in antibody engineering technologies, more and more therapeutic antibodies[7], including monoclonal antibodies (mAbs), antibody−drug conjugates (ADCs), bispecific antibodies (BsAbs), nanobodies, and others, have been developed. These can target and neutralize above disease-causing agents, such as self-antigens, cancer cells, and infectious agents[8-11]. So, it is necessary to track the *in vivo* dynamic changes of AAbs or therapeutic antibodies in case of diseases, which could benefit to predict their onset, and development, and even drive their precise treatment.

Frequent testing of clinically relevant antibodies allows for narrowing the delay between diagnosis and treatment, provides essential insights, and exhibits considerable clinical and therapeutic values for critical illnesses. However, the very low concentration of antibodies in patients' biofluids, together with the serious interferences of abundant serum proteins and other biomatrix components, will obscure the relationships between antibody levels and specific disease states, thus influencing their diagnostic or tracking accuracy[12]. To address these challenges related to sensitivity and non-specific interactions, several bioanalytical methods have been established, including enzyme-linked immunosorbent assay (ELISA)[13], Western blot[14], high-performance liquid chromatography (HPLC)[15], liquid chromatography/mass spectrometry (LC−MS)[16], protein/peptide-based microarrays[17], and so on. Although these techniques offer acceptable or excellent performances, they generally involve time-consuming pretreatments and procedures, expensive instruments, and skilled personnel, which strongly limit their clinical applications for point-of-care testing (POCT) or portable/wearable abilities. Over the past decades, electrochemical biosensors have attracted widespread attention and rapidly evolved because of their high speed, portability, miniaturization, and cost-effectiveness, which make them ideally suitable for biomolecule detection[18,19]. To contextualize electrochemical platforms within the broader diagnostic sensor landscape, a concise comparison is warranted. Optical biosensors, such as those based on surface plasmon resonance or fluorescence, typically offer exceptional sensitivity and label-free capabilities but often require sophisticated and costly instrumentation, are susceptible to sample turbidity or background fluorescence, and may face challenges in miniaturization for portability. Piezoelectric biosensors provide label-free, real-time mass detection but can be sensitive to environmental factors and may lack the sensitivity required for low-abundance antibodies. In contrast, electrochemical biosensors generally excel in terms of cost-effectiveness, ease of miniaturization and integration into portable devices, rapid response times, and lower power requirements, positioning them as strong candidates for decentralized testing. Benefiting from the progress in biotechnology, microfluidics, and nanomaterials, electrochemical biosensors are expected to develop into advanced portable diagnostic systems and monitoring devices for antibody analysis in case of critical illness[20,21]. Compared to optical biosensors, electrochemical biosensors have the potential to face the time-dependence and high-frequency challenge of antibody assays[22]. Despite the plenty of efforts that have been focused on the improvements of biorecognition elements, signal transduction strategies, and devices, electrochemical biosensors still have significant challenges for real-time measurements, including sensitivity, accuracy, stability, antifouling ability, immunogenicity, and high-throughput analysis (Fig. 1). In addition, the need for large-scale screening tests for antiviral antibodies in the human population during the COVID-19 pandemic also puts forward higher requirements on the cost, speed and throughput of detection methods as well as the portability of the detection equipment[23]. Moreover, most sensors are applied to a small number of real samples and tested only under laboratory conditions. The engineering of implantable and wearable devices for the *in vivo* monitoring of therapeutic antibodies remains an elusive goal (Fig. 1)[22]. Therefore, a comprehensive overview of the electrochemical sensors for the *in vivo* dynamic tracking of clinically relevant antibodies is needed.

In this review, we summarized and discussed the relationships and roles of AAbs and therapeutic antibodies in different diseases, the detection principles, strategies, core elements, and the development of electrochemical biosensors, as well as the clinical applications of novel biosensors. Furthermore, the review highlights the remaining challenges and opportunities for the advancement and prospects of electrochemical sensors in the context of clinically relevant antibodies.

2. Clinically relevant antibodies

2.1. Autoantibodies as biomarkers for disease diagnosis

AAbs against self-proteins are found not only in healthy people but can also be associated with a wide range of diseases, such as autoimmune diseases and cancers[24]. Their early detection and quantification are critical for clinical management, as AAbs often appear before overt symptoms and exhibit longer half-lives than

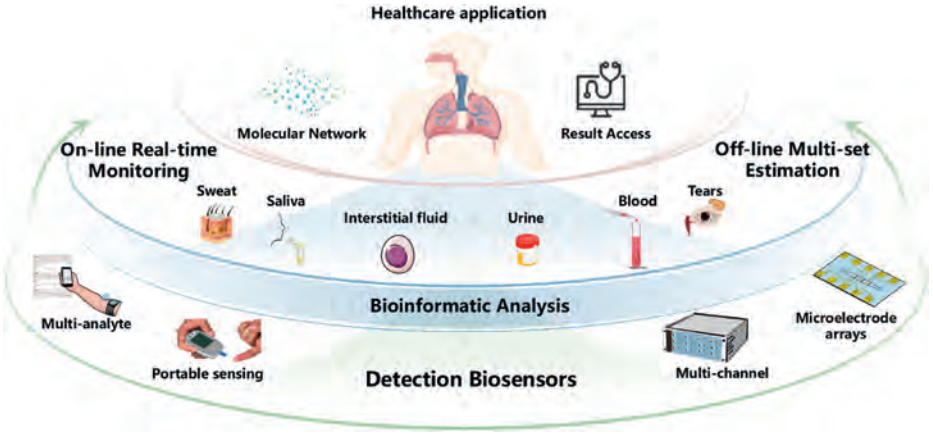

Figure 1 Multi-functional biosensors for on-line and off-line monitoring of diverse biomarkers in biological fluids for personalized healthcare.

their corresponding antigens in bodily fluids (*e.g.*, serum). These characteristics make AAbs ideal targets for electrochemical biosensors, which can leverage their stability and diseasespecificity to enable noninvasive, sensitive, and rapid detection (Fig. 2). Electrochemical platforms have been developed to monitor AAbs as predictive biomarkers for disease flares or treatment response, overcoming the limitations of conventional techniques (*e.g.*, ELISA) in cost, portability, and dynamic range. Thus, by focusing on AAbs as key analytes, electrochemical biosensors offer a promising tool for clinical diagnosis[25].

2.1.1. Role of AAbs in autoimmune disease diagnosis

Autoimmune diseases are a broad group of disorders in which an abnormal immune response attacks cells and tissues. This leads to chronic inflammation and thus damage to various organs and tissues, which results in a wide range of symptoms and complications[26]. To assess the risk and progression of autoimmune diseases, there are several biomarkers commonly used in clinical practice, such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complement proteins C3 and C4[27,28]. However, the specificity and/or sensitivity of these biomarkers is still unsatisfactory. In fact, in autoimmune diseases, patients' immune system cannot discriminate self-antigens from exogenous antigens, leading to the presence of abundant AAbs in serum and organs. In addition, studies have shown that these AAbs are associated with the disease itself or certain clinical phenotypes of one disease[29]. Each autoimmune disease exhibits a unique pattern of antibodies that can provide useful information for diagnosis and monitoring. For example, celiac disease (CD) can mainly be monitored by testing AAbs directed against tissue transglutaminase (tTG) and deamidated gliadin peptide (DGP)[30]. In patients with systemic lupus erythematosus (SLE), the anti-dsDNA level is highly related to the disease activity and is observed in about 70% of SLE patients. Anti-Sm antibodies can be detected in 30% of SLE patients with high specificity but low sensitivity[31,32]. Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of joints which causes the destruction of cartilage and synovial joints. The diagnostic testing sensitivity of anti-cyclic citrullinated peptide antibodies (anti-CCP Abs) was 64% in clinical studies, and the normal value was found to be below 20 EU/mL[33]. Other autoimmune diseases are indicated by different specific AAbs, such as myositis-specific autoantibodies (MSAbs) in myositis, anti-neutrophil cytoplasmic antibodies (ANCA) in inflammatory bowel disease (IBD), and ANCA-associated vasculitides[34,35].

2.1.2. Role of AAbs in cancer diagnosis

The incidence and prevalence of cancer are increasing worldwide due to population aging and growth, which has caused over 10 million deaths in 2022[33]. Therefore, the detection at early stages could help treatment and recovery, and reduce cancer mortality. During the earliest stages of tumorigenesis, it has shown that AAbs are also produced by the patient's immune system in response to proteins with altered structure and activated by abnormal expression of tumor-associated antigens (TAAs). The immune response to TAAs can be measured in the form of specific AAbs, which have been identified in virtually all types of cancers. Notably, AAbs appear several months or years before the clinical symptoms in different cancer types[36]. For example, tumor-suppressor p53 is mutated in up to half of all cancers, and the p53 AAbs have been significant biomarkers in cancer diagnosis, as reported for the first time in 1982[37,38]. Moreover, anti-prostate-specific antigen (PSA) AAbs could mediate anti-cancer effects and serve as a diagnostic biomarker in clinics. They have been approved by the FDA for prostate cancer diagnosis[39]. Most lung cancers (LCs) cases are diagnosed at advanced stages, primarily because earlier stages of the disease are asymptomatic. The wide distribution of AAbs has proved to be useful for early detection of LCs in general and non-small cell lung cancer (NSCLC) in particular. Studies have confirmed that a group of AAbs (p53, NY-ESO-1, CAGE, GBU4-5, Annexin 1, and SOX2) could help detect a high risk of LC and be identified in all histological types and stages[40]. Overall, since AAbs have proved to be effective and stable, more and more strategies for testing the presence of these endogenous serum biomarkers have been developed, which could even deduce cancer status before clinical manifestation.

2.1.3. Role of AAbs in viral disease diagnosis

In the last decades, the influenza H1N1 virus, the Middle East respiratory syndrome coronavirus (MERS-CoV), and the SARS-CoV virus have caused epidemics of respiratory infections, posing a significant threat to human health[41]. With this in mind, in the absence of either proven effective therapy or vaccine, diagnostic testing becomes an especially important tool, which can potentially help save lives by limiting the spread of the virus. Nucleic acid detection is currently the mainstream strategy for the

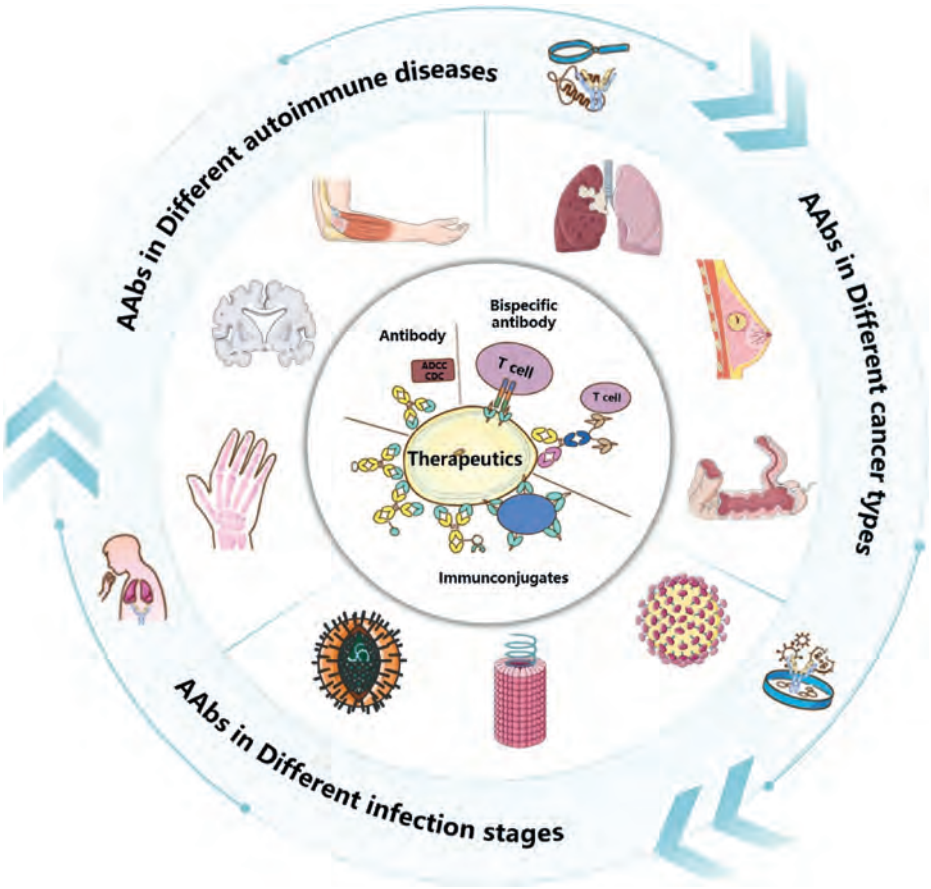

Figure 2 Clinically relevant antibodies for disease diagnosis or therapeutic.

diagnosis of patients with viral infections. Although it can help identify those who are infected, some inherent drawbacks cannot be neglected, including a delay of several days required to report results with high false-negative information[4,42]. Evidence suggests that the virus can be identified through the presence of antibodies generated in the organism, which may be present both during and after infection (*i.e.*, when the virus is no longer present). Moreover, recent studies[25] have reported that Immunoglobulins M (IgM) or G (IgG) against the immunogenic spike (S) and nucleocapsid (N) proteins of the severe acute respiratory Syndrome coronavirus 2 (SARS-CoV-2) were detected in patient blood for 3−6 days or even 8 days. Therefore, AAb detection combined with nucleic acid analysis could greatly improve the monitoring sensitivity and accuracy at the early, intermediate, and late stages. Besides, the high-performance detection of antiviral AAbs is crucial for understanding of the virus lifecycle. In particular, the detected levels of the antibody are related to its neutralization titer, which is particularly important for vaccine design[20]. In a word, antiviral AAbs have become very significant biomarkers in infectious diseases, and the timely detection of antiviral AAbs possesses epidemiological significance, which will be beneficial to track the occurrence, development, and prognosis of viral infection[43].

Despite the fact that AAbs have significant potential as biomarkers, their detection remains a key challenge in clinics. First, AAbs are difficult to detect clinically owing to trace-level concentrations (ng/mL) present before symptom onset[44]. Second, their corresponding target antigens or recognition elements may remain unidentified because these AAbs have not been associated with a specific disease. In general, it is complicated to construct a library and encode the ligands to identify a target. Third, clinical matrices exhibit high complexity, containing diverse biomolecules and abundant serum proteins that significantly interfere with AAbs detection. Overall, the development of novel bioanalytical technologies with high sensitivity and specificity is crucial for clinical AAbs diagnostics, particularly to enable efficient high-throughput screening for rapid response in public health settings.

2.2. *Antibody derivatives as therapeutic drugs for serious diseases*

Therapeutic antibodies have been used increasingly in recent years for targeted therapy[45]. To date, more than 100 therapeutic antibodies (including mAbs, antibody drug conjugates, BsAbs, nanobodies, etc.) have been approved and applied in various diseases, such as cancer, immune diseases, neurological disorders, infectious diseases, etc.[8]

MAbs have been demonstrated to be safe and effective treatments by multiple mechanisms that include direct modulation of the target antigen (Fig. 2), antibody-dependent cell-mediated cytotoxicity (ADCC), and complement-dependent cytotoxicity (CDC)[46]. Despite the increasing number of therapeutic mAbs in clinical use, their pharmacokinetic and pharmacodynamic properties are not fully clear. Severe and unexpected adverse effects are also observed in different clinical trials. For instance, infliximab targeting tumor necrosis factor alpha (TNF-α) has been

approved for the immunotherapy of Crohn's disease. Nevertheless, 10%−30% of patients with IBD show no initial clinical benefit from infliximab (primary non-response), or over 50% after an initially favorable outcome will lose response over time (secondary loss of response (SLR)[47,48]. For IBD patients, adalimumab was administered subcutaneously to the treatment. However, a proportion of IBD patients did not respond or exhibited SLR. Therefore, therapeutic drug monitoring (TDM) is commonly requested to assess the appropriate dose schedule for safe and effective therapeutic use.

ADCs take advantage of the specificity of mAbs to deliver potent cytotoxic drugs selectively to the desired cell population. Clear clinical benefits have been demonstrated with nearly 15 approved ADCs[49,50]. For example, DS8201 combines the humanized antibody trastuzumab with topoisomerase I inhibitor through a tetrapeptide-based cleavable linker for the treatment of patients with HER2-positive breast cancer, and it could effectively target tumor cells that express low levels of HER2[51]. Unfortunately, the greatest challenge to date in developing ADCs is still a therapeutic index far narrower than that expected. In fact, the toxicity profiles of ADCs are comparable to those of chemotherapeutics, with dose-limiting toxicities associated with the mechanism of activity of the cytotoxic warhead. For example, Gemtuzumab ozogamicin (GO) was the first approved ADC in 2000. It consisted of a humanized anti-CD33 monoclonal antibody linked to the DNA intercalator calicheamicin (CLM). Due to increased mortality and no observed survival benefit, it was withdrawn from the U.S. market in 2007. However, an adjustment of the dosing strategy and sequence of administration allowed the re-approval of GO by the FDA in 2017. In the new approval, the safety of a fractionated low dose of 3 mg/m^2 on Days 1, 4, and 7 was demonstrated[52]. To date, the clinical trials of 92 ADC candidates out of over 260 have been terminated because of intolerable toxicity and insufficient therapeutic efficacy[53]. Moreover, it has been reported that after administration to patients, less than 1%−2% of an ADC reaches the tumor in humans, while the remainder potentially causes unwanted off-target toxicity and undergoes complex biotransformations (including linker cleavage, payload deconjugation or metabolism, and antibody modifications)[54]. Therefore, the dynamic tracking of ADCs in the blood circulatory system is of high interest for their TDM in early-stage development and clinical applications[55].

Owing to the complex disease pathogenesis, mAbs or ADCs directed against a single target antigen are often unable to show sufficient therapeutic effect. BsAbs or bispecific ADCs (BsADCs), which have been gaining increasing interest in recent years, have been designed to target tumor cells and effector cells simultaneously, triggering the cytotoxicity of effector cells towards tumor cells[56,57]. Like other drugs used to treat severe diseases, therapeutic BsAbs may cause different side effects. It has been reported that Abs against therapeutic BsAbs appear in blood or that cytokine release-related symptoms occur during treatment. Depending on their production method and structure, BsAbs can differ in the number of antigen-binding sites, geometry, half-life in blood serum, and effector functions. The development of BsAbs exhibiting excellent antigen-binding properties presents an obvious challenge from the beginning. It is necessary to build analytical platforms to help design BsAbs that are more stable and produce lower toxicity and immunogenicity to patients.

There are still several challenges to be faced for the monitoring of therapeutic antibodies. First, the widespread reliance on ELISA for therapeutic antibodies monitoring belies its fundamental challenges: unstable immunoreagents, poor inter-batch reproducibility, nonspecific interactions, and protracted analysis time. These issues demand alternative analytical approaches. Second, biological challenges compound these technical issues, particularly limited samples volume and high-concentration endogenous IgG interference (7−18 mg/mL). The analytical system must achieve broad dynamic range coverage to quantify therapeutic antibodies across clinically relevant concentrations, while maintaining sensitivity at both lower and upper quantification limits. Additionally, the platform must enable high-throughput analysis to support real-time clinical decision-making. Third, besides the drug concentration monitoring, the tracking of the dynamic changes of the drug-to-antibody ratio of ADCs and BsADCs in the blood circulation system and their biotransformation (linkers, small molecule drugs, and antibodies) is particularly important. Thus, the dynamic *in vivo* monitoring of antibodies is crucial for the efficacy[43] and risk assessment in the early development stages, while revealing clinical pharmacokinetic behavior and clearance mechanisms[58]. Electrochemical biosensors could address these gaps by offering rapid, low-volume testing for ADCs/BsAbs amid serum interference and multiplexing capability to track drug-to-antibody ratios and biotransformations simultaneously.

3. Electrochemical biosensors

Among the technologies utilized in diagnostic and TDM, electrochemical biosensors have been developed as a trending technology with their unique advantages of rapid response, high sensitivity, miniaturization, cost-effectiveness, and user-friendly operation, enabling specific detection of targets in complex fluids (such as whole blood) or even *in vivo* environments. The electrochemical biosensor is an integrated device that provides analytical information, either quantitative or semiquantitative, using a biorecognition strategy involving an electrochemical transducer[59]. In essence, a fully functional electrochemical biosensor system requires the integration of three critical components: (a) a biorecognition element to specifically interact with the target; (b) a signal transducer to convert a measurable signal from the specific interaction between the target and the biorecognition element, and (c) an electronic system for the data management and output[60]. These biosensors not only possess the excellent specificity of the biological receptor, but also take profit from the high sensitivity and low detection limit of electrochemical detectors[61]. In particular, they can be miniaturized and combined with portable, implantable, or wearable devices, fulfilling the need for real-time, continuous, and reagentless detection for safe monitoring[22]. Therefore, electrochemical biosensors are increasingly regarded as a highly promising advancement for antibody detection beyond traditional analytical instruments, with considerable potential for widespread application to the analysis of various clinical samples. The different components of these electrochemical biosensors will be systematically described in this section.

3.1. Biorecognition elements for electrochemical biosensors

To ensure the specificity of the electrochemical biosensors, biorecognition elements (Fig. 3A), serving as core functional components, can precisely recognize target antibodies and correlate their concentration to a measurable electronic signal[62,63]. They are primarily categorized into antigens, aptamers, peptides, peptide

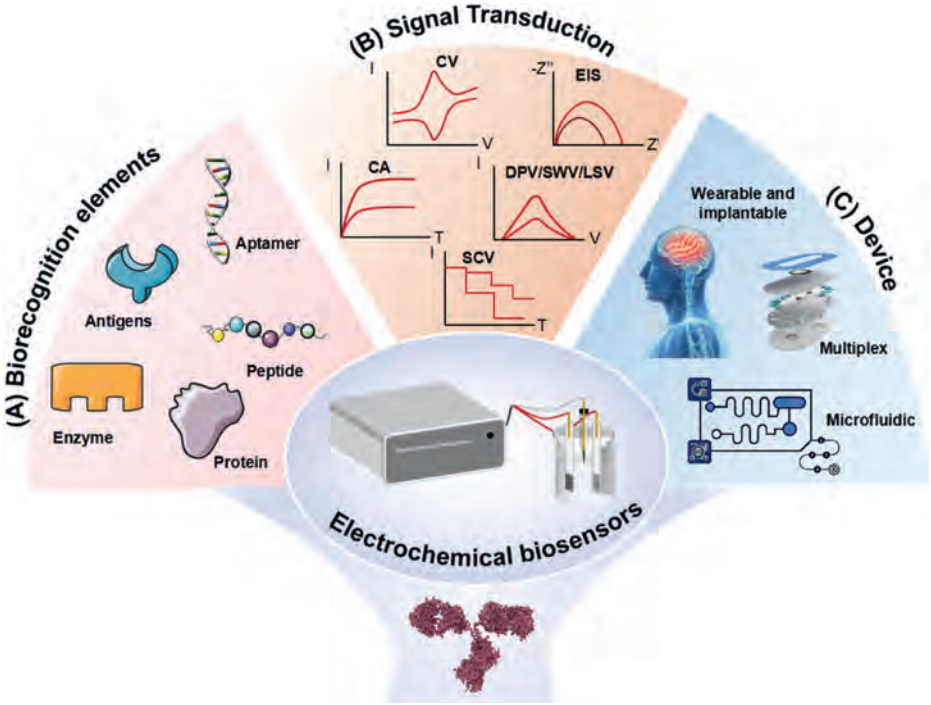

Figure 3 Schematic illustration of electrochemical biosensors. (A) The corresponding bioreceptors such as aptamers, antigens, peptides, enzymes and proteins are biorecognition elements for the specific detection of antibodies. (B) The different classes of signal transduction strategies based on electrochemical detection techniques (CV, EIS, CA, DPV, SWV, LSV, SCV). (C) Sensing platforms for antibody detection based on multiple devices.

nucleic acids (PNAs), and molecularly imprinted polymers (MIPs). Based on the applications of these recognition elements, three types of electrochemical biosensors have been systematically outlined.

3.1.1. Immuno-based electrochemical biosensors

The detection mechanism of immunosensors is predicated on the principle of antibody−antigen specificity. Target antibodies bind to antigens (including enzymes, proteins, DNA, hormones, bacteria, viruses, small molecules, etc.) immobilized on electrode surfaces, inducing measurable electrochemical signal alterations. To enhance sensitivity, enzyme-labeled secondary antibodies amplify signals *via* substrate conversion. Given the inherent diversity of AAbs (different epitopes of the antigen may produce different types of AAbs in terms of structure and modification), the immune-based recognition is the most appropriate detection mode for AAbs, offering superior specificity and sensitivity compared to alternative recognition elements. Antigen immobilization strategies critically influence biosensor performance. Simple physical adsorption (*e.g.*, electrostatic interactions[64], covalent conjugation methods, biotin-avidin chemistry[65], EDC/NHS (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/*N*-hydroxysuccinimide) reaction[66], azide reaction[67], disulfide bond modification[68] and so on) improve directed immobilization. It is worth noting that improper covalent immobilization may hinder the antigen binding sites and affect the activity of the antigen, and even influence the performance of the biosensor[69]. Recent advances employ aptamers/peptides/polymers to selectively bind specific sites on proteins, thereby exposing their active regions and assisting protein orientation[69]. Concurrently, various nanomaterials[64], magnetic nanomaterials[70], multi-walled carbon nanotubes (MWCNTs)[71], and specialized forms of graphene[72] have been engineered to expand the surface area for antigen immobilization, resulting in improved immobilization efficiency and optimized electron transfer at the electrode interface[69]. However, the development of these biosensors is accompanied with several intrinsic challenges: (1) High-purity antigens incur costs and batch variability, particularly for antiviral antibody detection requiring recombinant antigens; (2) Antigens are susceptible to denaturation under fluctuating environmental conditions (*e.g.*, temperature, pH), potentially impairing antibody-binding capacity. (3) Overcrowded antigen immobilized on the electrode surface result steric hindrance, which weaken the signal strength and affect the binding interactions with the target antibodies. Collectively, these challenges could compromise the accuracy and reliability of immuno-based electrochemical biosensors for antibody detection.

3.1.2. Peptide-based electrochemical biosensors

To address the limitations of immunoelectrochemical sensors, a series of peptide-based electrochemical sensors have been developed for antibody detection. Peptides exhibit superior recognition capabilities by emulating the behavior and function of the antigen, and they can be efficiently screened using advanced techniques, such as phage display[73], combinatorial chemistry[74], and virtual screening[75]. The unique physical and chemical properties of peptides, including facile synthesis, chemical versatility, high stability, and excellent biocompatibility, make them highly suitable for applications in electrochemical biosensors[76-78]. Furthermore, the small weight, compact size and flexible conformation of peptides effectively reduce steric hindrance during molecular recognition, which in turn promotes more precise interactions with

the target analytes. Therefore, our group designed diverse epitope mimetic peptides (HH24 peptide[79], m-EDPW[80], CN14 peptide[81], HN19 peptide[77], HT25-cyclopeptide[82]) for precise recognition of trastuzumab, rituximab, hIgG (Human immunoglobulins G) and ADCs, and these mimotope peptides exhibited desirable binding affinities. In addition, peptides are promising antifouling materials to solve the biofouling problem of biosensor interfaces in contact with complex biomatrices. For example, Luo's group[83,84] developed a series of antifouling peptides (polyhydroxyproline helical peptide, self-assembled loop-closed peptide, and three-in-one peptides) to minimize the impact of nonspecific adsorption, thus enhancing the performance and reliability of peptide-based sensors in complex biological fluids. Interestingly, the cyclic structure of peptides and the addition of non-natural amino acids (D-amino acids) could improve the stability of peptide-based biosensors and reduce the possible enzymatic degradation[85,86]. Furthermore, peptides can be engineered into highly ordered supramolecular assemblies with multi-functionalization, boosting binding affinities and enhancing stability, which is suitable for biosensor construction[87,88]. However, due to their simple structure, the chemical modifications of peptides (such as conjugation with redox reporter) may influence their binding to the target antibody. Additionally, baseline fluctuations in voltammetry and the drifting of the capacitance current may be caused by the uncontrolled stability of the secondary structure in the peptide sequence[21]. Moreover, most of the peptide sequences for the binding to antibodies are derived from the corresponding antigens, and there is still a lack of effective guidance for the design of peptides with higher specificity. In general, the potential of peptide-based electrochemical biosensors for antibody detection is considerable, and it will continue to be developed with the advancement of peptide structure design and functionalization.

3.1.3. *Aptamer-based electrochemical biosensors*

Aptamers, as another prominent category of recognition elements, are typically selected from libraries of oligonucleotides through an *in vitro* process, such as the systematic evolution of ligands by exponential enrichment (SELEX: an *in vitro* selection technique that identifies high-affinity nucleic acid aptamers (DNA/RNA) capable of binding specific molecular targets (proteins, small molecules, cells)[89]. Following several rounds of screening and careful probe design/modification, it is possible to discover an aptamer with high specificity and affinity towards the target antibody. Currently, several specific affinity aptamers have been successfully screened as specific ligands to therapeutic antibodies (trastuzumab[90], rituximab[91], bevacizumab[92], ranibizumab[93]). Due to their nucleic acid constitution, the aptamers offer distinct advantages including low cost, uniform synthesis and customizable modification. The screened aptamer CH1S-3 could even distinguish between native and heat-treated trastuzumab, identifying such minor conformational change that is difficult to detect through immunological recognition[90]. Compared to macromolecular antigens, aptamers possess excellent thermal stability, recovering their active conformation and keeping their affinity after thermal denaturation. Moreover, the structure of aptamers plays a critical role in the signal transduction and the sensor design scheme. The generation of a measurable signal is associated with the conformational change resulting from the binding of the aptamer to the antibody. For example, Nagata et al.[94] designed a redox probe-modified bivalent anti-idiotype aptamer to detect bevacizumab. The interaction between two recognition regions of the aptamer and bevacizumab leads to conformational change of the aptamer, thus causing a change in electron transfer efficiency. These aptamer-based electrochemical biosensors are helpful to achieve real-time analysis and reagentless, label-free detection[95]. Furthermore, benefiting from complementary base pairing, aptamers are able to integrate more complex systems such as a reaction with DNA to achieve more sensitive and specific detection of the target antibody[96]. Although the long-term stability of aptamer-based electrochemical biosensors in complex biological environments remains unresolved, their potential for continuous at-home health monitoring is driving the development of applications to the monitoring of therapeutic antibodies.

3.2. *Signal transduction for electrochemical biosensors*

In clinical settings, antibodies serve as biomarkers and therapeutic drugs, making a significant contribution to the early diagnosis, monitoring of disease progression and prognostic evaluations. However, challenges such as low concentrations of antibodies, the presence of highly homologous proteins and a complex environment in serum require improved sensitivity and accuracy in electrochemical detection. To achieve maximum electrochemical sensor performance, it is necessary to gain a comprehensive understanding of the signal mechanism. Therefore, the electrochemical detection techniques, the detection methods and the signal transition modes will be discussed in the next two sections.

3.2.1. *Electrochemical detection techniques*

Electrochemical detection techniques (Fig. 3B) are divided into the following groups based on the signal generation and measurement methods: (1) Voltammetry is based on the measurement of the current response across a variable voltage range. According to the way of varying the applied potential, voltammetry can be classified into linear sweep voltammetry (LSV, constant rate potential), cyclic voltammetry (CV, forward and reverse potential), differential pulse voltammetry (DPV, fixed-amplitude pulses superimposed on a linear potential), square wave voltammetry (SWV, symmetrical square waveform of potential) and alternating current voltammetry (ACV, small-amplitude sinusoidal alternating current potential superimposed on a linear direct current potential ramp). Distinct from the unidirectional scanning in LSV, CV enables comprehensive investigation of both oxidative and reductive processes, making it particularly valuable for initializing electrochemical studies for new systems and obtaining information about complicated electrode reactions. The pulse-based techniques DPV and SWV demonstrate superior performance in protein quantification applications through minimizing the influence of background current and enhancing detection sensitivity. Notably, DPV exhibits enhanced resolution for closely spaced redox peaks, which is essential for accurately quantifying specific proteins in mixtures. Meanwhile, SWV offers distinct advantages in rapid data acquisition and heightened sensitivity, rendering it particularly advantageous for high-throughput analyses requiring fast scan rates. ACV, by resolving the alternating current component in-phase and out-of-phase with the applied voltage, offers exceptional sensitivity and the unique ability to separate background currents from reaction currents. These techniques generally involve the employment of redox tags, such as ferrocene, methylene blue (MB), thionin and so on. (2) Amperometry detect target analytes through continuous current monitoring under constant voltage conditions, includes chronoamperometry (CA), pulse amperometry (PA), and chronocoulometry (CC). CA establishes a direct linear relationship between current intensity and

the concentration of electroactive species, enabling precise quantification of proteins and making it particularly useful for kinetic analysis. This characteristic has led to its widespread application in antibody detection systems where enzymatic reactions generate electroactive products. PA applies a sequence of short, constant potential pulses (often with cleaning and detection steps) and measures the current near the end of each pulse. This approach significantly reduces fouling effects by periodically desorbing reaction products or contaminants from the electrode surface, enhancing stability and extending sensor lifetime, which is particularly advantageous in complex biological matrices or for continuous monitoring. In contrast, CC employs current-time integration to determine the cumulative quantity of electroactive species, which correlates with total protein content. A distinctive advantage of CC lies in its capacity to differentiate between faradaic processes (electron transfer reactions) and non-faradaic contributions (capacitive/adsorptive effects), rendering it particularly valuable for investigating protein adsorption/desorption dynamics at electrode interfaces. (3) Electrochemical impedance spectroscopy (EIS) requires the application of an amplitude sinusoidal alternating current excitation signal to a working electrode, to sensitively detect protein-surface binding interactions through monitoring changes in charge transfer resistance and capacitance. As a non-faradaic technique, EIS typically employs surface-immobilized bioreceptors that specifically recognize target analytes, subsequently forming sterically hindered complexes at the electrode interface. The recognition complex effectively obstructs the diffusion pathway of exogenous redox mediators and thus increases charge transfer resistance, generating a decreased current in comparison to the unbound recognition elements. Compared with voltammetry/amperometry, EIS offers a low-cost, label-free, and minimally invasive method for protein quantification. (4) Potentiometry operates by measuring the potential difference between a working electrode (functionalized with biorecognition elements) and a reference electrode under zero or negligible current conditions. When a target protein binds to the recognition element on the sensor surface, it induces a change in the interfacial potential, which is then transduced into a measurable electrical signal. Therefore, it can realize direct, real-time analysis and label-free protein detection, which are easily miniaturized and integrated into portable or wearable devices. (5) Photoelectrochemistry (PEC) synergistically integrates the photoactivity of semiconductor materials with electrochemical transduction mechanisms. Upon light irradiation, photoactive nanomaterials (*e.g.*, TiO_2, Zinc oxide (ZnO), or quantum dots (QDs)) undergo electron-hole pair separation, leading to the generation of a measurable photocurrent. This photoelectrochemical response demonstrates linear proportionality to analyte concentrations, thus providing a convenient platform for analytical applications with inherent signal amplification capabilities. (6) Electrochemiluminescence (ECL) is a photon-emission phenomenon where electrogenerated species undergo electron-transfer reactions to form excited states that emit light upon relaxation. In protein bioassays, target proteins are specifically captured by immobilized recognition elements. When subjected to appropriate electrochemical potentials, the luminophore system (typically $[Ru(bpy)_3]^{2+}$ complexes or luminol derivatives) undergoes redox cycling to produce characteristic photon emissions, which are quantitatively detected using photomultiplier tubes or semiconductor photodetectors. The emitted light intensity exhibits a linear correlation with target protein concentration across clinically relevant dynamic ranges. Both PEC and ECL sensors involve light irradiation for the electrocatalytic reactions, which merges the merits of both optical and electrochemical techniques. The dual-mode signal transduction mechanism confers superior signal-to-noise ratios (S/N) and exceptional sensitivity. Notably, ECL has achieved widespread commercial adoption in automated immunoassay systems. However, it is to a great extent dependent on the optical-assistant electrocatalytical efficiency, which is not trivial to modulate. Furthermore, the analytical performance is critically dependent on the photoelectrocatalytic conversion efficiency at electrode interfaces, which presents significant optimization challenges regarding material engineering and surface modification strategies. (7) Organic Electrochemical Transistors (OECTs) leverage ionic modulation of an organic semiconductor channel. A gate voltage controls ion flow into the channel, which amplifies changes in source-drain current. This inherent amplification provides high sensitivity for label-free biosensing, operates at low voltages, and is compatible with flexible substrates, making them ideal for wearable diagnostics and real-time monitoring in biological fluids. These diverse techniques offer distinct advantages for improving the sensitivity, accuracy, and efficiency of electrochemical detection of proteins, meeting the demands of complex biological analysis[12].

3.2.2. Electrochemical detection strategies and signal change modes

Based on the abovementioned electrochemical biosensors and detection techniques, a series of sophisticated electrochemical detection strategies have been developed. According to the detection principle, these strategies are classified into four categories: (1) In direct detection, the antibody is directly captured by a bioreceptor immobilized on the electrode to induce an electrical signal change without additional reagents. It is obvious that the advantages of simplicity and rapidity of direct measurement are most suitable for development into POCT and TDM. However, this also places higher demands on the affinity of antibodies and recognition elements, as well as on the detection interface resistance to non-specific adsorption. (2) The indirect detection involves the detection of signals from unbound bioreceptors or a labeled probe related to the antibody. The process converts the target antibodies to alternative molecules, facilitating enhanced signal amplification. (3) The sandwich detection is a representative strategy of immuno-based electrochemical biosensors, in which the target antibody is captured between two different receptors. By targeting different recognition regions of the antibody, the sandwich detection substantially reduces the potential for nonspecific adsorption, which greatly improves the detection sensitivity and specificity. (4) The competitive detection relies on the difference in binding affinities between the recognition element and the antibody towards hybridized sequences. While the competition assay is effective, there are inherent risks associated with the potential failure of the analysis due to the close affinity between the recognition element-antibody pair and the aptamer-competitive strand pair. Therefore, the success of such an assay configuration necessitates a careful design of the competitive strand, with particular attention to the rational adjustment of its affinity.

Then, according to the mode of signal change, the electrochemical detection strategies can be classified into “turn-on”, “turn-off”, and “ratiometric” detection. When the target antibody is present, as binding events obstruct electron transfer, the current signal is decreased. This detection method is referred to as the “turn-off” mode. However, external factors may affect the

transmission of the signal, resulting in the decline of the signal. Therefore, the more selective "turn on" mode possesses a lower S/N. The "ratiometric" method effectively mitigates the influence of systemic errors and electrochemical instrument fluctuations on the results by incorporating a calibration signal, enhancing the accuracy of the measurements. To facilitate reader comprehension, we have systematically reviewed and categorized the recent advancements in electrochemical sensors for antibody detection, based on detection strategies and signal transduction mechanisms (Table 1[37,64-68,70,71,81,93,94,96-162]).

3.3. Devices for electrochemical biosensors

Electrochemical biosensors have emerged as a promising technology in personalized medicine, offering rapid and highly sensitive target detection with minimal sample requirements. These advantages make them ideally suited for *in vitro* diagnostics (IVD) and POCT. A case in point is the widespread adoption of blood glucose meters. However, such examples of successful commercialization remain rare. In this section, we will provide some insight into the potential development directions of electrochemical biosensor devices (Fig. 3C) in response to the challenges associated with antibody detection.

3.3.1. Integrated and microfluidic devices

To achieve high sensitivity in antibody detection and to mitigate the interference of matrix effects, whole blood samples often require pre-treatment processes, such as centrifugation, separation, and dilution. However, these labor-intensive procedures pose the risk of negatively impacting the accuracy and reproducibility of the assay. Consequently, integrating the multiple sample processing steps into a single micro-scale unit holds the significant advantage of avoiding human-induced variability and allows for fully automated, rapid detection. Microfluidic chips, in particular, have emerged as a prominent platform for such integration. By leveraging the advancements in electrochemical sensor miniaturization, these chips have been extensively utilized in the detection of infectious diseases such as COVID-19, hepatitis, malaria, and tuberculosis[163-166]. For instance, the Nanomix Corporation has developed a microfluidic chip capable of detecting the SARS-CoV-2 nucleocapsid antigen, completing the entire detection process in just 15 min[163]. The development of these integrated and microfluidic devices represents a convergence of multiple disciplines, including medicine, bioengineering, chip fabrication, and materials science. With continued collaboration across these fields, integrated microfluidic devices are expected to reach new heights of sophistication, offering a more advanced approach to antibody detection.

3.3.2. Multiplex devices

Almost all autoimmune diseases are linked to a specific set of complementary biomarkers that serve as diagnostic references. However, the assessment of AAbs as standalone biomarkers is inadequate for providing a comprehensive reflection of disease progression. Moreover, the advent of companion diagnostics has emphasized the importance of the detection of specific biomarkers to ensure the safety and efficacy of antibody therapies. To address these challenges, it is imperative to develop multi-target analysis. The strategies are generally classified into two types: the first involves the immobilization of multiple recognition elements on a single electrode. Although this approach permits simultaneous detection, it is susceptible to cross-interference and entails strict specifications regarding the design and immobilization of the recognition elements. The second strategy employs an array of electrodes, where each electrode is functionalized with a distinct recognition element corresponding to a specific target. This approach is more practical and stable, thereby reducing the risk of cross-interference. In light of these strengths, a multitude of research teams have proceeded to develop multiplex electrochemical biosensors, building upon their own prior work. Arévalo et al.[167] constructed an electrochemical platform for the quadruple determination of different AAbs produced against extractable nuclear antigens (ENAs). Chen's team prepared microneedle-coupled epidermal sensors for multiplexed electrochemical detection of kidney disease biomarkers[168]. Li's group[169] designed a series of hand-held and integrated tubular tip-like sensing platforms for biomarker semi-automated multiplexed assay. Indeed, the integration capabilities inherent to microfluidic devices offer significant potential for the development of multiplex detection systems. For example, a portable paper-based microfluidic platform was designed for multiplexed electrochemical detection of human immunodeficiency virus and hepatitis C virus antibodies in serum[125]. As metabolomics continues to progress, it is anticipated that further multiplex sensors will be developed, thereby enhancing the accuracy and precision of disease diagnostics.

3.3.3. Wearable and implantable devices

Traditional blood sample analysis is intermittent, requiring frequent clinic visits and invasive blood draws. In contrast, emerging wearable and implantable biosensing devices offer continuous remote monitoring of dynamic changes in target biomarkers and therapeutic drugs, which is undoubtedly one of the most promising and meaningful researches. However, these equipments face a series of challenges including the development of flexible electrodes, biocompatibility and long-term stability of sensors, lifespan and calibration issues. Wearable microneedle patches provide a minimally invasive and painless method for collecting analytes from the skin for disease diagnosis and long-term TDM[170]. However, the S/N of the electrochemical detection is poor due to the weak conductivity, easy degradation and instability of the microneedles, which reduces the accuracy of the monitoring. Non-invasive detection using alternative biofluids such as sweat, saliva, and tears offers another promising pathway for wearable devices. Innovations in this field include smartwatches, ultra-light conductive nanofibers, responsive DNA hydrogels and so on[171]. However, secreted fluids such as sweat, saliva and tears are filtered from blood and interstitial fluid (ISF) to a significant extent, resulting in markedly lower analyte concentrations, requiring higher sensitivity for detecting a relevant signal. Additionally, the majority of wearable equipments are designed to detect small molecules such as cortisol, and it represents a serious challenge to achieve effective real-time monitoring of macromolecular proteins. Therefore, it is necessary to develop implantable devices to obtain precise diagnosis and customize drug treatment plans by monitoring the unique metabolic characteristics of individual patients. Implantable devices are introduced partially or fully into the body through surgical procedures to record physiological or biochemical information, providing closer contact with tissues compared to wearable sensors. Despite their considerable potential, implantable devices still require further exploration before widespread clinical application. It is recommended that research efforts be concentrated on the improvement of the fundamental elements of electrochemical sensors, as well as on the advancement of both implantable and

Table 1 Recent advancements in electrochemical biosensors for antibody detection.

Electrochemical biosensor	Target	Recognition element	Detection strategy	Detection techniques	Signal change	Detection range	LOD	Ref.
Immuno-based electrochemical biosensors	Anti-P53 autoantibodies	Human P53 antigen	Direct detection	EIS	Turn-on	1.0−1000 ng/mL	130 pg/mL	66
				SWV	Turn-off	0.1 pg/mL−10 ng/mL	0.088 pg/mL	64
				DPV	Turn-off	10−1000 pg/mL	3.2 pg/mL	67
			Sandwich detection	CA	Turn-on	0.0875−7 U/mL	0.08 U/mL	65
					Turn-on	0.02−14 U/mL	0.02 U/mL	97
				DPV	Turn-on	10−500 pg/mL	0.01 pg/mL	98
				ECL	Turn-on	0.1 fg/mL−0.01 ng/mL	0.03 fg/mL	99
		HaloTag fusion proteins	Sandwich detection	CA	Turn-on	1.1−5 U/mL	0.34 U/mL	37
		Concanavalin A	Sandwich detection	SWV	Turn-on	5.0 pg/mL−0.5 μg/mL	1 pg/mL	100
	Anti-tissue transglutaminase antibodies	Tissue transglutaminase	Sandwich detection	CA	Turn-on	0.26−6.9 μg/mL	0.26 μg/mL	68
						/	390 ng/mL	101
				CV		/	/	102
	Antigliadin antibodies	Gliadin	Sandwich detection	CA	Turn-on	0−0.75 μg/mL	20 ng/mL	103
						/	46 ng/mL	104
						0−10 μg/mL	33 ng/mL	105
	Anti-DGP antibodies	DGP	Sandwich detection	CA	Turn-on	/	/	106
	Antigliadin (AGA) and anti-tissue transglutaminase (tTG) antibodies	Gliadin and tissue transglutaminase	Sandwich detection	CA	Turn-on	0−100 U/mL	3.16 U/mL (AGA IgA), 2.82 U/mL (AGA IgG), 2.45 U/mL (tTG IgA), 2.95 U/mL (tTG IgG),	71
	anti-dsDNA antibodies	dsDNA	Sandwich detection	CA	Turn-on	1−200 IU/mL	0.3 IU/mL	107
						3.4−100 U/mL (hIgG), 5.0−100 U/mL (hIgM), 5.9−100 U/mL (hIgA), 1.9−100 U/mL (Three hIgs)	1.01 U/mL (hIgG), 1.49 U/mL (hIgM), 1.77 U/mL (hIgA), 0.56 U/mL (Three hIgs)	108
						/	10 μg/mL	108
						/	8 μg/mL	109
	Anti-nuclear autoantibodies	Rabbit thymus extract	Sandwich detection	PA	Turn-on	/	/	110
	Islet autoantibodies	Human islet autoantigen	Sandwich detection	ECL	Turn-on	/	/	111
	Anti-Toxoplasma gondii antibodies	T. Gondii antigen	Sandwich detection	CA	Turn-on	0−200 U/mL	0.012 U/mL	112
	Clostridium tetani antibodies	Clostridium tetani	Sandwich detection	CA	Turn-on	0.01−0.25 IU/mL	0.011 IU/mL	113
	Antibodies against human growth hormone	Human growth hormone	Sandwich detection	ECL, PA, CV	Turn-on	0.098−0.695 nmol/L (ECL), 0.098−1.11 nmol/L (PA, CV)	0.061 nmol/L (ECL), 0.027 nmol/L (PA) 0.056 nmol/L (CV)	114
	Glutamate decarboxylase antibodies	Glutamate decarboxylase	Direct detection	ECL	Turn-off	0.30−50 ng/mL	0.1 ng/mL	115
	Alpha-synuclein autoantibodies	Alpha-synuclein	Direct detection	EIS	Turn-on	75 ng/mL−1.5 μg/mL	8.2 ng/mL	116
	IgM-rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP Abs)	Fc fragment of native human IgG protein and cyclic citrullinated peptide	Sandwich detection	CA	Turn-on	3−300 IU/mL (RF), 10−1000 IU/mL (anti-CCP Abs)	0.8 IU/mL (RF), 2.5 IU/mL (anti-CCP Abs)	117

(*continued on next page*)

Table 1 (*continued*)

Electrochemical biosensor	Target	Recognition element	Detection strategy	Detection techniques	Signal change	Detection range	LOD	Ref.
	Anti-myelin basic protein autoantibodies	Myelin basic protein	Sandwich detection	CA	Turn-on	0.05−50 ng/mL	0.016 ng/mL	70
	Anti-DNP antibodies	Dinitrophenyl-ε-amino-n-caproic acid (DNP)	Indirect detection	SWV	Turn-on	/	/	118
	Antibodies against His6-H5 HA	Recombinant His-tagged hemagglutinin (His6-H5 HA)	Direct detection	EIS	Turn-on	4−20 pg/mL	2.1 pg/mL	119
	Anti-hemagglutinin H1 antibodies	Recombinant His-tagged hemagglutinin (His6-H1 HA)	Direct detection	SWV	Turn-off	Vaccinated mice sera diluted from 1×10^9 to 1×10^8 times	/	120
	Anti-dengue antibodies	Dengue virus (DENV)	Direct detection	EIS	Turn-off	/	/	121
		Dengue Virus 2 NS1 glycoprotein	Direct detection	EIS	Turn-on	10^{-12}−10^{-5} g/mL (PBS), 10^{-11}−10^{-5} g/mL (plasma)	10^{-12} g/mL	122
	Anti-EDIII and anti-NS1 antibodies	Domain III of the envelope protein (EDIII) and fragment of a non-structural (ΔNS1) protein	Direct detection	EIS	Turn-on	8.7−5.8 mg/mL (Anti-EDIII) 9.6−5.3 mg/mL (Anti-NS1)	53 fg/mL (Anti-EDIII) 17 fg/mL (Anti-NS1)	123
	Hepatitis C core antibodies	Dual-affinity yeast chimera	Sandwich detection	CV	Turn-on	4−250 nmol/L	2 nmol/L	124
	Anti-human immunodeficiency virus (Anti-HIV) and anti-hepatitis C virus (Anti-HCV) antibodies	HIV p24 core antigen and HCV core antigen	Sandwich detection	CA	Turn-on	/	300 pg/mL (Anti-HIV) 750 pg/mL (Anti-HCV)	125
	Measles-specific IgG	Measles-antigen	Direct detection	EIS	Turn-on	10 ng/mL−5 μg/mL	/	126
	Anti-Leishmania infantum antibodies	Recombinant L. infantum antigens	Direct detection	PEC	Turn-off	1−300 nmol/L	0.41 nmol/L	127
		Soluble antigens of Leishmania infantum	Direct detection	EIS	Turn-on	1:20,000−1:50 diluted canine sera	/	128
	Pseudorabies virus (PrV) antibodies	Pseudorabies virus (PrV)	Sandwich detection	ECL	Turn-on	1 pg/mL−50 ng/mL	0.40 pg/mL	129
	Porcine circovirus type 2 (PCV2) antibodies	Porcine circovirus type 2 (PCV2)	Sandwich detection	SWV	Turn-on	$1{:}10^2$−$5{:}10^7$ diluted serum	/	130
	Porcine epidemic diarrhea virus (PEDV) antibodies	Porcine epidemic diarrhea virus (PEDV)	Sandwich detection	ECL	Turn-on	0.1−5000 pg/mL	0.05 pg/mL	131
	Anti-SARS-CoV-2 spike protein antibodies (IgM and IgG)	SARS-CoV-2 spike protein	Sandwich detection	CA	Turn-on	/	/	132
				CA	Turn-on	10.1 ng/mL−60 μg/mL (IgG), 1.64 ng/mL−50 μg/mL (IgM)	10.1 ng/mL (IgG), 1.64 ng/mL (IgM)	133
			Direct detection	DPV	Turn-off	1 fg/mL−1 μg/mL	0.3 fg/mL	134
				SWV	Turn-off	1−1000 ng/mL	0.96 ng/mL (IgG), 0.14 ng/mL (IgM)	135
	Anti-SARS-CoV-2 antibodies (IgG, IgA and IgM)		Direct detection	EIS	Turn-on	0.41−4.81 BAU/mL	0.4 BAU/ml	136

	SARS-CoV-2 immunoglobulin G (IgG)		Direct detection	OECT	Turn-on	10 fmol/L−100 nmol/L	10 fmol/L	137
	Anti-SARS-CoV-2 antibodies	SARS-CoV-2 spike S1-his recombinant protein	Direct detection	SWV	Turn-off	0.1−1000 ag/mL	0.01 ag/mL	138
	Clones of recombinant monoclonal antibodies (4G6, 7F10 and 1A6)	SARS-CoV-2 recombinant nucleocapsid protein (rN)	Direct detection	CV, DPV, PA	Turn-off	/	0.04, 0.02, 0.05 μg/mL (CV, 4G6, 7F10, 1A6), 0.08, 0.92, 0.10 μg/mL (DPV, 4G6, 7F10, 1A6), 0.54, 0.56, 0.81 μg/mL (PAD, 4G6, 7F10, 1A6)	139
	Adalimumab	Anti-Ada fragment of antigen bindin	Sandwich detection	CA	Turn-on	0.1−2 μg/mL	15.5 ng/mL	140
	Rituximab	EGFR protein	Indirect detection	CA	Turn-on	1 pmol/L−0.1 μmol/L	1 pmol/L	141
	Bispecific antibody (contain one binding site that binds the EGFR protein and the other that targets MUC1 protein)	EGFR protein	Indirect detection	CA	Turn-on	/	0.4 pmol/L	
Aptamer-based electrochemical biosensors	Anti-P53 autoantibodies	Aptamer of anti-P53 autoantibody	Direct detection	EIS	Turn-off	0.1 pg/mL−0.1 ng/mL	0.1 pg/mL	142
	Rituximab	Double stranded salmon sperm DNA (ds-DNA)	Direct detection	DPV	Turn-off	7−60 μmol/L, 60−300 μmol/L	0.56 μmol/L	143
	Bevacizumab	Aptamer with an MB tag (5′-HS−SH-$(CH_2)_6$-TMBTTTTTTTT GCGGTTGGTGG TAGTTACGTTCGC-3′)	Indirect detection	SWV	Ratiometric	0.025−2.5 μg/mL	6.5 ng/mL	144
		Anti-idiotype bivalent aptamer modified with a redox probe	Direct detection	SWV	Turn-off	1−100 nmol/L	9.1 nmol/L	94
		Chimeric hairpin DNA aptamer	Direct detection	SWV	Turn-off	1 pmol/L−1 μmol/L	0.37 pmol/L	145
	Trastuzumab	Thiolated DNA aptamer for trastuzumab (5′-HS-$(CH_2)_6$-TTTTTTTTTGCG GTTGGTGGTAGTT ACGTTCGC-3′)	Indirect detection	SWV	Turn-on	0.05−50 ng/mL	71.5 pg/mL	146
	Ranibizumab	Aptamer (5′-AGGGAAGAGA AGGACATATGAT GGGGGCTAGTATC GACGTCTGCCTAT GTTGTTAGTCGGT GTTGACTAGTACA TGACCACTTG-3′)	Direct detection	EIS	Turn-on	25−100 nmol/L	25 nmol/L	93

(*continued on next page*)

Table 1 (*continued*)

Electrochemical biosensor	Target	Recognition element	Detection strategy	Detection techniques	Signal change	Detection range	LOD	Ref.
Peptide-based electrochemical biosensors	Anti-cyclic citrullinated peptide antibodies	Citrullinated-modified peptide (ITIH3$^{542-556}$ Cit)	Direct detection	EIS	Turn-on	1/2−1/64 serum dilution	/	147
	Aquaporin-4 antibodies	E loop of extracellular AQP4	Direct detection	DPV	Turn-off	0.01−20 ng/mL	6.2 pg/ml	148
	Anti-p24 antibodies	Antigenic peptide epitope from the HIV-1 p24 capsid protein	Direct detection	ACV	Turn-off	5−100 nmol/L	5 nmol/L	149
		EAA, SG-EAA, EK-EAA peptides	Direct detection	ACV	Turn-off	/	0.5 nmol/L (EAA), 1 nmol/L (SG-EAA, EK-EAA)	150
		21-Residue peptide epitope (DRY-MB) from the HIV-1 p24 antigen	Direct detection	ACV	Turn-off	/	1 nmol/L	151
	HIV-1/2 antibodies	Epitopes from the Env glycoprotein, gp4 and the Env glycoprotein, gp36	Sandwich detection	DPV	Turn-on	1−1000 ng/mL	1 ng/mL	152
	4E10 antibodies	NWFDIT epitope of protein gp41	Indirect detection	SWV	Turn-on	1−100 nmol/L	/	153
	Anti-hemagglutinin antibodies	Coiled-coil peptide with integrated HA-antibody specific peptide (YPYDVPDYA)	Direct detection	EIS	Turn-on	1 pg/mL−100 ng/mL	1 pg/mL	154
	Anti-Wuchereria bancrofti antibodies (anti-Wb)	Synthetic peptide (NENQSWTDKGCFCEG) containing the epitope Wb/ALT2-A5	Sandwich detection	CV	Turn-on	/	5.0 μg/mL	155
	Anti-circumsporozoite protein (Anti-CSP) and anti-thrombospondin related anonymous protein (Anti-TRAP) antibodies	Peptide CSP210-247	Direct detection	EIS	Turn-on	95.9 fg/mL−17 ng/mL	/	156
	Anti-hemagglutinin antibodies	Peptide (9-residue epitope in HA protein)-PNA strands	Indirect detection	SWV	Turn-off	/	2.9 nmol/L	157
	Cetuximab	Peptide (12-residue epitope in EFDR protein)-PNA strands	Indirect detection	SWV	Turn-on	/	/	96
	Anti-HIV antibodies	Peptide (13-residue epitope in protein p17 from HIV)-PNA strands	Indirect detection	SWV	Turn-on	/	/	96

	Anti-HA antibodies	Peptide (9-residue epitope in human influenza hemagglutinin protein)-PNA strands	Indirect detection	SWV	Turn-on	/	/	96
	Trastuzumab	Cysteine-containing peptide mimotope CH19 (CGSGSGSQLGPY ELWELSH)	Direct detection	EIS	Turn-on	0−16 μg/mL	0.22 μg/mL	158
	Rituximab	CD20 epitope mimetic peptide CN14 (CGSGSGSWPRWLEN)	Sandwich detection	SWV	Turn-on	0.1 pmol/L−1000 nmol/L	56.4 fmol/L	159
				EIS	Turn-on	0.1−50 μg/mL	35.26 ng/mL	81
PNA- based electrochemical biosensors	Anti-tissue transglutaminase antibodies	tTG functional PNA	Direct detection	SWV	Turn-off	0.01−10 U/mL	0.01 U/mL	160
	Anti-gp41 antibodies	Epitope-PNA chimeras	Direct detection	SWV	Turn-off	/	/	161
MIP- based electrochemical biosensors	Anti-HIV-1 antibodies	Human IgG-MMIPs	Sandwich detection	ECL	Turn-on	1:20,000−1:50 dilution ratios	1:60,000 dilution ratio	162

wearable technologies, with the objective of developing more effective and reliable approaches for continuous monitoring.

4. Applications of electrochemical biosensors to clinically relevant antibody detection

4.1. Electrochemical biosensors for the detection of AAbs

4.1.1. Detection of AAbs in autoimmune diseases

There has been a significant increase in autoimmune disease diagnoses over the past few years, whose symptoms are extremely vast, implying difficulties for physicians to establish a diagnosis[26]. The quantitative detection of AAbs in biological fluids provides valuable clinical information for early diagnosis of autoimmune diseases. In this context, electrochemical biosensors emerge as a promising diagnostic alternative, capable of detecting autoimmune markers during pre-symptomatic stages.

RA is an inflammatory autoimmune disease that causes the destruction of cartilage and synovial joints. Early diagnosis of RA is extremely important for applying suitable treatment to prevent irreversible joint damage. Anti-CCP Abs have proven to be RA biomarkers with 94% specificity. In clinical practice, the cut-off value of the anti-CCP Abs level has been set at 20 IU/mL, and a level above 20 IU/mL suggests the possibility of RA. For the detection of anti-CCP Abs, citrullinated vimentin, citrullinated α-enolase, or citrullinated fibrinogen can be used as antigens, but synthetic Cyclic citrullinated peptides (CCPs) are more commonly employed. The high-efficiency immobilization of CCPs can be achieved on iron oxide nanoparticles (IONPs) obtained by green synthesis for the early identification of RA. Due to the biocompatibility and high electroconductivity of IONPs, the voltammetric sensor exhibited a dynamic range of 4−250 pg/mL and a LOD of 19 pg/mL[172]. To expand the detection range, an anti-CCP Ab-based immunosensor was fabricated using molybdenum disulfide (MoS_2) polyaniline (PANI) as the matrix of the screen-printed electrode (SPE) (Fig. 4A). The matrix incorporating AuNPs and CCPs on the electrode is highly effective to entrap anti-CCP Abs, and the analysis allowed for the quantification in the linear range from 0.25 to 1500 IU/mL, with a LOD of 0.16 IU/mL[173]. Although these biosensors show high sensitivity with a proper LOD, they require complex modification steps and extra labeling probes to collect the data, which significantly narrow down their clinical applicability. Impedance-based biosensors on the surface of interdigitated electrodes (IDEs) allow miniaturization, label-free measurement, and low cost for anti-CCP Ab detection. In this study, densities and pattern distributions of AuNPs colloids onto IDEs were identified, thus increasing the impedance signals and resulting in a LOD of 0.12 ng/mL (Fig. 4B). With the high mass transfer obtained with this kind of sensor, the real-time monitoring of anti-CCP Abs could be achieved over a period of 1 h, which would make rapid bedside monitoring feasible for early detection of RA[174].

Affinity peptides with high specificity and excellent biocompatibility are extremely useful for anti-CCP Ab detection. First, chimeric peptides that contain fibrin and filaggrin domains have proven to be highly specific to anti-CCP Abs, enabling more reliable results for early diagnosis of RA[175,176]. Then, a novel peptide corresponding to inter-alpha-trypsin inhibitor-3 (ITIH3), has been validated as antigen for RA diagnoses with sensitivities of 94.0%−100% and specificities of 79.6%−98.4%. Furthermore, this peptide immobilized onto the nanogold surface of the

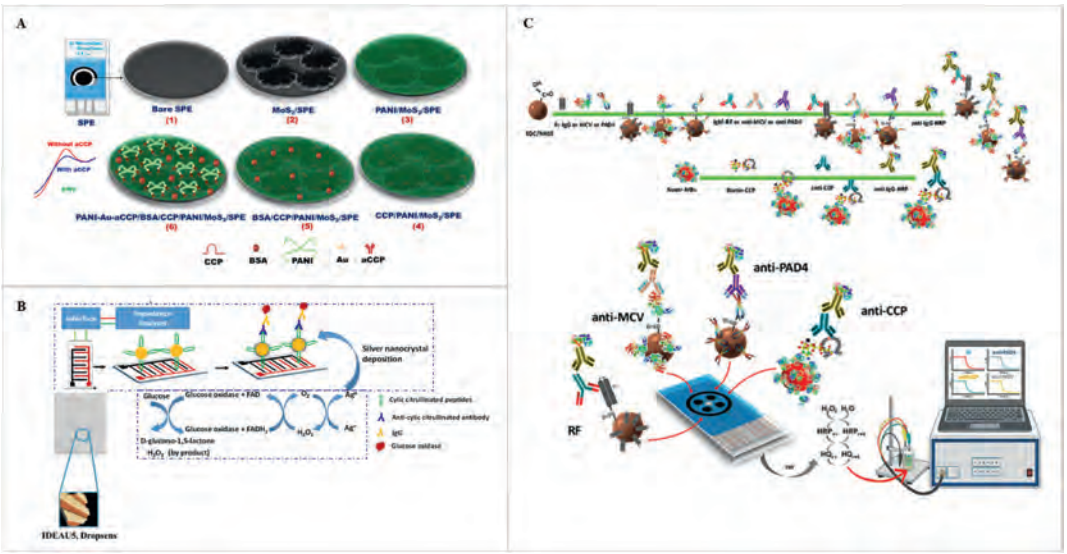

Figure 4 Electrochemical biosensors for autoimmune diseases antibody analysis. (A) Schematic representation of electrochemical nanobiosensor for anti-CCP Ab detection. Reprinted with the permission from Ref. 173. Copyright © 2021 Elsevier. (B) Schematic representation of IDE sensors for anti-citrullinated peptide antibody detection. Reprinted with the permission from Ref. 174. Copyright © 2020 Elsevier. (C) Schematic representation of immunoplatform suitable for the simultaneous determination of four RA autoantibodies biomarkers. Reprinted with the permission from Ref. 178. Copyright © 2023 MDPI.

electrochemical chip was used to confirm anti-CCP Ab values in serum samples of RA patients. The proposed method showed better sensitivity and was able to be a complement to the current conventional anti-CCP ELISA[147]. Besides, the M-12 peptide, which mimics carbonic anhydrase III (CAIII), has also been employed to develop detection devices. Herein, 3-hydroxybenzoic acid was electropolymerized onto graphite electrodes for the further adsorption of the M-12 peptide. The M-12 peptide functionalized sensor could discriminate between positive and negative RA patients according to DPV changes[177].

Beyond the exploration of a single biomarker, the combined use of anti-CCP Abs with the rheumatoid factor (RF) was found to be more useful for the diagnosis of early RA. Dual screen-printed carbon electrodes (SPCEs) and functionalized magnetic beads were used as substrates for the immobilization of Fc fragments of IgG and biotinylated CCP, which are specific recognition elements for RF and anti-CCP Abs, respectively. Under the optimized conditions, the proposed sensor displayed good sensitivity, and the quantification of both targets could be completed with about 2 h. In very early RA, the combined detection of anti-CCP Abs and RF will significantly increase the sensitivity compared with the detection of anti-CCP Abs alone[117]. Pingarrón's group[178] further reported on a magnetically assisted electrochemical immunoplatform for the simultaneous determination of four RA biomarkers: RF IgM, anti-PAD4, anti-CCP Abs, and anti-MCV Abs. The developed platform was applied to the determination of the biomarkers in human serum of twenty-two patients diagnosed with RA and four healthy individuals, and it provides emergent biomarker information within a short time and affordable cost (Fig. 4C). It is competitive with commercial ELISA kits available only for the single determination of these biomarkers, which is suitable to meet the demands of current clinical practice for assisting in the diagnosis of RA patients.

Celiac disease (CD) is a gluten-induced autoimmune enteropathy, which will lead to an abnormal intestinal mucosa and malabsorption-related problems. The characteristics of serological changes include the appearance of anti-gliadin Abs and anti-tissue transglutaminase (anti-tTG) Abs. Therefore, the detection of these serological AAbs could provide diagnostic information about CD with high sensitivity and specificity. Initially, the employment of self-assembled monolayers containing thiols has been demonstrated to be efficient for the immobilization of the antigen on a gold electrode surface. O'Sullivan's group[68,101,104] designed a series of electrochemical detection platforms for anti-tTG Abs using thiol-based surface chemistry. All these platforms were found to give highly reproducible results using real patients' samples, with clinically relevant dynamic linear ranges and LODs. Supramolecular interactions are also introduced for the construction of stability surfaces. The use of cyclodextrin and polypyrrole-cyclodextrin-coated surfaces offers an unconventional route for the immobilization of gliadin. Thanks to the highly hydrophilic characteristics of cyclodextrin, the nonspecific interactions on the electrode surface can be greatly reduced. Remarkably, the developed cyclodextrin-based supramolecular biosensor was successfully applied to the detection of anti-AGA Abs directly in serum samples of CD patients[103,105].

Due to the low abundance of AAbs in serum, nanohybrid materials have been used to fabricate ultrasensitive sensors through AAb targeting and signal amplification. QDs-based biosensors were constructed for the detection of anti-tTG Abs. Tissue-transglutaminase was attached to the solid support, followed by labeling with CdSe/ZnS QDs. This signal probe released Cd^{2+} *in situ* by voltammetric stripping, generating a current enhancement. Such a sensor mechanism allows sensitive detection over the range from 3 to 100 U/mL with a LOD of 2.4 U/mL in serum[179,180]. Besides, Gupta et al.[181] hybridized graphene quantum dots (GQDs) and polyamidoamine on AuNPs that were embedded in MWCNTs to detect anti-tTG Abs. This electrochemical immunosensor was able to provide a very low LOD of 0.1 fg anti-tTG Ab per 6 μL of serum using DPV. Although

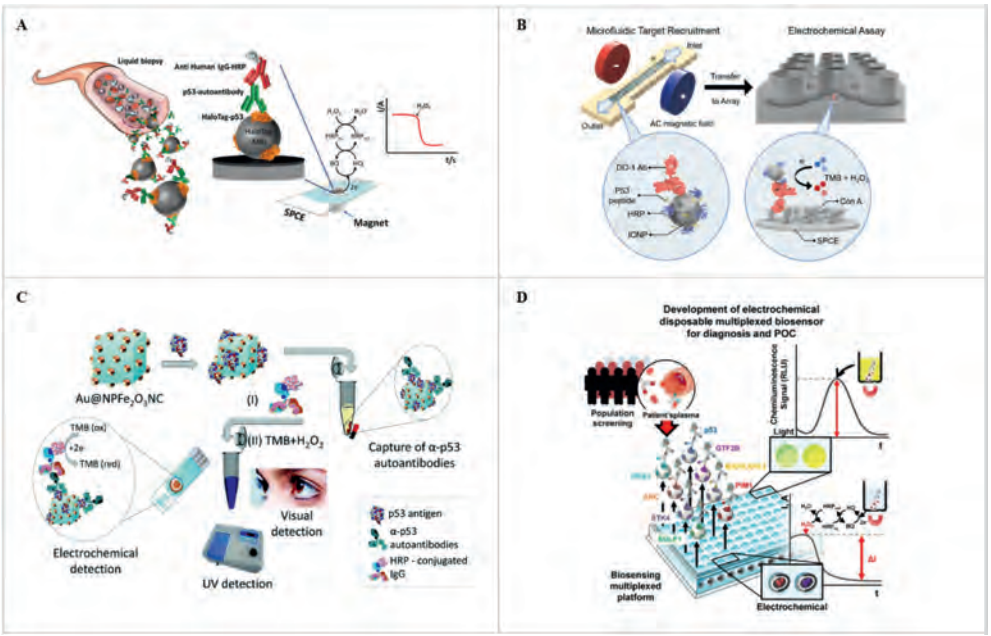

Figure 5 Illustration of AAbs sensing in multi-modal. (A) Schematic representation of immunosensing platform for determination of p53-specific autoantibodies. Reprinted with the permission from Ref. 37. Copyright © 2016 American Chemical Society. (B) Schematic representation of antigen-mimic nanoparticles for anti-p53 antibody quantification. Reprinted with the permission from Ref. 100. Copyright © 2024 American Chemical Society. (C) Schematic representation of nanocubes as capturing agents for tumor-associated autoantibody analysis. Reproduced with the permission from Ref. 97. Copyright © 2017 Royal Society of Chemistry. (D) Schematic representation of a multiplexed electrochemical immunoassay capable of detecting autoantibody presence for colorectal cancer detection. Reprinted with the permission from Ref. 193. Copyright © 2020 Ivyspring International Publisher.

current works still focus on only one biomarker detection, a disposable electrochemical dual immunosensor has been constructed for the simultaneous detection of anti-AGA and anti-tTG Abs in CD patient serum. The CV signals were formed by the anodic redissolution of enzymatically generated silver. This biosensor is one of the most promising analytical tools for CD diagnosis[71,106]

The autoimmune condition multiple sclerosis (MS) is one of the most known inflammatory neurological diseases that attack the central nervous system (CNS). Anti-myelin basic protein antibodies (anti-MBP Abs) appearing in MS patient's serum and cerebrospinal fluid (CSF) have been regarded as a biomarker of the disease progression. Real-Fernandez et al.[182-184] fabricated an electrochemical biosensor for specific antibody direct detection in patients' sera without any preliminary electrode modification through an antigenic probe. This probe, a glycopeptide analogue of Fc-CSF114 (Glc) bearing a ferrocenyl moiety, showed efficient recognition for anti-MBP Abs. These work have the main analytical advantage of being rapidly implemented in microfluidics and possibly automated for point-of-care screenings. A label-free immunosensor was constructed by Derkus et al.[185] for the anti-MBP Ab detection using alginate-TiO_2 nanocomposite material. Impedance measurements revealed that the electron transfer resistance was proportional to the anti-MBP Ab concentration, and the immunosensor had a low response time of 79 s with the alginate-TiO_2-MBP electrode, which is highly promising for addressing the requirements of POCT. A magnetic microbeads (MBs)-based bioanalytical platform was also developed by Pingarron's group[70] for the determination of anti-MBP Abs by preparing MB immunocomplexes in homogeneous solution. After only a single incubation step, the complexes were labeled with a secondary antibody conjugated to the horseradish peroxidase enzyme (HRP). Finally, H_2O_2 was added as the enzyme substrate, using hydroquinone as redox mediator. Thus, the immunosensor response was achieved due to the electrochemical reduction of the quinone generated by the enzyme reaction. More importantly, this platform exhibited a rapid and sensitive detection of anti-MBP Abs in 75 min, with an LOD of 0.054 ng/mL. It is worth noting that the detection is 2 times faster and 62.5 times more sensitive than that of the commercial ELISA. Besides the above examples, there are also many other AAbs that play a crucial role in the pathogenesis of autoimmune diseases. For example, antinuclear antibodies (ANAs) are a sensitive indicator of SLE and related diseases[186]. The detection of these AAbs would be greatly beneficial for disease monitoring and treatment. Seeking new methodologies and technologies for the sensitive, rapid, and low-cost screening of AAbs will lead to the improvement of diagnostic efficiency.

4.1.2. Detection of AAbs in cancers

Specific AAbs can be readily measured in cancer patients' sera due to the inherent stability and long half-life of immunoglobulins in circulation, which will promote assay reproducibility and reliability[187-191]. The representative p53 protein is a tumor-suppressor, present in a mutated form in up to half of all cancers[192]. Moreover, the anti-p53 Abs with a positive predictive value ($\geq$0.20 ng/mL) are associated with the overexpression of the tumor suppressor p53[65]. Although their induction mechanism in the body's immune system is unclear, anti-p53 Abs serve as a prevalent biomarker for early cancer screening and diagnosis (Fig. 5A)[37,193,194].

In recent years, several electrochemical biosensors have been developed for the detection of anti-p53 Abs in various cancer patients. The ideal analytical performances of these biosensors depend on their recognition elements and immobilization methods. First, human p53 protein as a classical recognition element can be fixed on the electrode surface through physical adsorption or covalent immobilization. While physical adsorption is simple and reagent-free, the adsorbed protein (*e.g.*, p53) may desorb in protease-rich environments or have uncontrolled orientation. Covalent immobilization overcomes these issues by forming stable bonds. For example, Adeniyi et al.[66] designed an electrochemical detection platform by step-to-step functionalization, including the modification of the gold electrode with phenylethylamine (PEA) through electrochemical grafting to form stable gold carbide on the electrode, and further covalent attachment of p53 protein for the sensitive detection of anti-p53 AAbs. This biosensor showed a concentration-dependent impedimetric response and a wide linear range (1.0−1000 ng/mL) with a LOD of 130 pg/mL for anti-p53 Abs. This LOD was 3 times lower than that of commercial ELISA (390 pg/mL). In particular, this immunosensor could be applied in early-stage diagnosis and screening of different tumor-associated AAbs. More recently, chitosan (CS) with its abundant reactive amine groups, enable stable antigens immobilization on the electrode surface while mitigating interferences from endogenous biothiols to reduce false-positive. A glassy carbon (GC) electrode was functionalized with a CS/$Ni(OH)_2$NPs nanocomposite to immobilize p53 antigen, then coupled with an HRP-labeled secondary antibody for electrochemical signal readout. Under optimal conditions, the LODs obtained with this biosensor for anti-p53 Abs were significantly improved, reaching 0.001 pg/mL (DPV mode) and 0.007 pg/mL (EIS mode), respectively[98]. Besides, electropolymerization was exploited to fabricate protein-immobilizing nanomaterials, offering advantages including minimized crosslinker interference, simplified purification, and enhanced synthesis efficiency. For instance, poly(3,4-ethylenedioxythiophene) (PEDOT) has been considered a state-of-the-art coating due to its ability to provide surfaces with high conductivity, chemical stability and antifouling capability. Cruz-Pacheco et al.[67] combined PEDOT films with cerium oxide (CeO_2) NPs stabilized with cyanopropyltriethoxysilane on screen-printed gold electrodes (SPAuE). Covalent bonds between the cyano groups from the CeO_2 NPs and the p53 antigen amine groups could be formed without the addition of cross-linking agents. The p53/CeO_2/PEDOT nanocomposite-decorated SPAuE demonstrated tremendous potential to detect anti-p53 Abs in human serum with a linear range from 10 to 1000 pg/mL and a LOD of 3.2 pg/mL. Despite these advanced immobilization methods, clinical anti-p53 Abs detection still face significant challenges, including limited ligand surface density, pronounced steric hindrance, and slow target diffusion in complex biofluids. An ultrasensitive on-chip quantification method for anti-p53 Abs was reported by Kang et al.[100], in which antigen-mimicking peptide-coated magnetic nanoparticles were used to construct the electrochemical sensors (Fig. 5B). This novel platform using peptide-based nanoparticles demonstrated 400% higher sensitivity than that observed with p53 protein under equivalent preparation and analytical conditions. Hence, the potential of magnetic nanoparticles that could achieve dynamic target recruitment in the presence of a magnetic field is immense in biosensors for AAb monitoring.

More importantly, the multi-modal detection of AAbs (colorimetric/electrochemical/fluorescence) enhance both sensitivity and selectivity while adapting to diverse analytical requirements. For example, a new class of gold-loaded superparamagnetic ferric oxide nanocubes (Au-NPFe_2O_3NC) was synthesized, which can be used as nanoenzymes with enhanced peroxidase-like activity for the electrochemical and colorimetric detection of AAbs in patient plasma[65]. Using this diagnostic platform, the assay of anti-p53 Abs in plasma can differentiate stage I patients with ovarian cancer from noncancerous healthy controls. As expected, both colorimetric and electrochemical methods showed very good inter-assay reproducibility (RSD <5%) for the analysis of patients with differential expression patterns of anti-p53 Abs at different stages. Rapid and low-cost diagnostic tools are required based on the detection of AAbs in complex biofluids. Superparamagnetic nanoparticles enable highly specific anti-p53 Ab detection in serum by leveraging magnetic enrichment and purification (Fig. 5C), effectively minimizing biological noise from nonspecific adsorption. They provide a blueprint for AAb measurements in whole blood[97]. In previous works, although multiplexed immunoassays were achieved using multi-electrodes, they were subject to repeated modifications, time-consuming operation steps, and large sample volume. So far, the HaloTag technology (Haloalkane Dehalogenase-Based Protein Tagging Technology: an engineered protein tagging system derived from a bacterial haloalkane dehalogenase)-based electrochemical immunosensing platform was designed for anti-p53 Ab detection as a diagnostic tool of colorectal cancer (CRC). Using magnetic microcarriers as solid supports, the platform was modified with covalently immobilized HaloTag fusion proteins for selectively capturing different AAbs (Fig. 5D). The biosensing platform was applied to the analysis of AAbs against TAAs described for the first time in plasma samples from healthy asymptomatic individuals, patients with high-risk of developing CRC, and patients already diagnosed with colorectal, lung or breast cancer. It demonstrated an improved discrimination between CRC patients and controls compared to an ELISA-like luminescence test. The proposed strategy employs cost-effective, high-throughput instrumentation to simultaneously detect eight AAbs in minimal plasma volumes, enabling early diagnosis of CRC[193]. Therefore, as ideal candidates for high-throughput and multiplexed detection, future electrochemical sensors should evolve toward automated, portable systems with minimal sample needs to achieve accurate, multiplexed cancer monitoring through parallel AAb profiling.

4.1.3. Detection of AAbs in viral infections

Viruses are proficient in fast dispersal and therefore represent enduring threats to the worldwide public health. Electrochemical biosensors can be deployed as point of care devices to help medical staff make quick triage and handle decisions when analyzing patients' treatment responses[195-197]. The current section provides information about the most common viruses and their specific antibodies that can be used for the diagnosis and monitoring of these diseases.

4.1.3.1. Human immunodeficiency virus (HIV). HIV has been an epidemic since the 1980s and it is divided into two types, including HIV-1 and HIV-2[198]. Anti-HIV antibodies (Anti-HIV Abs) are generated after infection, which is of great importance for the evaluation of antiviral therapies[199]. To date, several electrochemical

biosensors based on enzymes[200], MIPs[162], affinity peptides[201-203], and PNAs[153] were designed for the specific detection of anti-HIV-1 Abs in patients. First, a fast, simple, and reliable HIV diagnostic method was constructed *via* the combination of allosteric enzymes and coulometry. The presence of anti-HIV Abs results in an increase (above 50%) of enzyme activity, and this platform has allowed for the monitoring of anti-HIV-1 Abs in serum samples within only 1 h[200]. Second, MIPs can replace virus antigen proteins to precisely capture anti-HIV-1 Abs due to their high affinity, stability, and ease of chemical modification. Therefore, sandwich-type ECL immunosensor-based magnetic MIPs (MMIPs) were designed for the detection of anti-HIV-1 Abs. The LOD of this platform was 50 times lower than that of an ELISA method[162]. Nevertheless, the specificity of MIPs is always limited by the size and shape of the template molecules and thus may result in false positive signals. Third, some new peptides exhibiting high stability against denaturation, satisfactory affinity, specificity and easy modification, can be employed as good recognition elements for anti-HIV-1 Ab detection[201]. A direct biosensor was developed through an antigenic peptide epitope (TINEEAAEWDRVHP) from the HIV-1 p24 capsid protein. This peptide can be further labeled with MB *via* "click" chemistry, which exhibited a characteristic peak at a reduction potential in ACV[149]. In addition, Kelley's group[152] has devoted itself to developing an electrochemical ELISA system based on HIV antigenic peptides such as LAVERYLKDQQLLGIWGCSGKLICT. This peptide-based biosensor exhibited good selectivity, sensitivity and detected the AAbs in the range of 0.001−1 μg/mL with a low LOD of 1 ng/mL (6.7 pmol/L) for both HIV-1 and HIV-2. Finally, the excellent anti-fouling abilities of short thiolated oligonucleotides[151] used as passivating diluents or short hydrophilic peptides such as EKEKEK[150] incorporated into the peptide probe, were exploited to prevent interferences from the complex biomatrices. Moreover, E-DNA scaffold sensors with higher affinity and specificity were designed, which comprise a rigid nucleic acid "scaffold" attached *via* a flexible linker to an electrode and modified on its distal end with a redox reporter and an antibody-binding epitope as recognition element[161,204]. This reagentless, single-step electrochemical approach was used to achieve anti-HIV Ab quantification, exhibiting thermal stability and chemical stability against nucleases and proteases in blood. Compared to ELISA and lateral flow assays, the novel biosensor achieved the required 90% sensitivity and 100% specificity, in quantitative detail. The platform can also achieve the detection of anti-HIV Abs in 2 steps (less than 12 min) without requiring a fixed read-out time window. More interestingly, a peptide-mediated electrochemical steric hindrance hybridization assay was developed for the rapid one-step detection of specific anti-HIV-1 Abs directly in blood plasma samples due to the high specificity and rigidity of the PNA backbone. This biosensor executes a reagent-less process without signal drift or decay and can achieve a 10 times higher signal response than those obtained with other electrochemical biosensors[153]. In a pilot clinical study, Mckendry's group[205] engineered small, portable and dual-channel biochips on biosensors to diagnose HIV in 133 patient samples. Testing for individual biomarkers revealed sensitivities of 100% (anti-gp41) and 64.5% (anti-p24) with a combined 100% specificity in 5 min. This technology combines all of the benefits of current rapid HIV tests based on lateral flow (cost, time to result, simplicity of use), supporting faster access to treatment and counselling for those who test both positive and negative.

4.1.3.2. Influenza viruses. Influenza viruses could induce annual epidemics and occasional pandemics, which will have a high level of mortality. This will pose major challenges for vaccine protection and environmental control due to the high seasonal variability[206]. Viral biosensors offer exciting alternatives to traditional diagnostic assays for different analytes such as viral nucleic acids (DNA and RNA), viral proteins, intact viral particles, and antibodies generated by the patient's immune response against the virus[207]. Typically, viruses can mutate to change their coat proteins or have a high similarity of coat proteins. The presence of virus-specific antibodies in serum is directed against a surface coat protein of the influenza virion, hemagglutinin (HA). Thus, HA-specific antibodies are associated with the risk of influenza infection in populations. Recently, a peptide epitope was derived from the amino acid sequence 98−106 (YPYDVPDYA) of human influenza virus HA, and then this peptide epitope was employed to construct a microelectrode array platform for the monitoring of influenza virus HA-specific antibodies[154]. Besides, Radecka's group[119,120,208] has devoted significant efforts to the development of protein-based sensors for the detection of Abs against avian influenza virus H5N1. In detail, a redox-active layer consisting of a dipyrromethene (DPM)-Cu (II) complex was proposed for a more stable and precisely oriented immobilization of the histidine-tagged HA (H5) of the virus. The sensitivity of the immunosensor was four orders of magnitude higher than that of ELISA. Likewise, a voltammetric biosensor was reported for the detection of Abs against anti-swine influenza virus H1N1. Based on the His-tag chemistry, the DPM-Cu (II) monolayer could maintain the biological activity of the corresponding HA (H1) on the gold electrodes. The unprecedented sensitivity for anti-influenza virus Abs was found to be several orders of magnitude (10^7) higher than that of ELISA. Overall, this DPM-Cu (II) monolayer could be a universal material to achieve extended dynamic ranges for the detection of antibodies against different viruses.

4.1.3.3. Dengue. Dengue is an infection caused by the dengue virus (DENV) of the Flavivirus family. As the transmission occurs through a mosquito (Aedes aegypti) bite, effective prevention is very important to avoid dissemination[209]. Conventional ELISA only allows for the detection of dengue toxin antibodies after infection for 3−5 days but not in early-stage cases[210]. Some interesting sensor technologies have been developed to detect AAbs so far. For example, carbon nanotube (CNT) layers functionalized with the DENV 2 non-structural 1(NS1) glycoprotein were developed for the reliable and highly sensitive monitoring of anti-DENV 2 NS1 Abs. Such a platform achieved excellent sensitivity in plasma and linearity in concentration range from 10^{-11} to 10^{-5} g/mL[122]. Moreover, polymeric films derived from 4-aminobenzoic acid, 4-hydroxybenzoic acid or the corresponding benzamides were immobilized on the graphite SPAuE surface to increase the conductive efficiency and combined with EIS technology to detect the anti-NS1 glycoprotein Abs in serum samples of immunized mice[211]. However, serological tests for specific antibodies in remote areas are still challenging, while portable devices enable rapid detection for health monitoring. Sampietro et al. described a microfluidic platform based on the differential impedance technology for the detection of anti-DENV Abs. In this sensor, a synthetic peptide (DRGWGNGCGLFG) was designed as recognition element to mimic the antigenic determinant of Dengue full envelope protein. A solution of biotinylated goat anti-rabbit IgG was chosen as secondary antibody to bind to anti-DENV Abs and a suspension of streptavidin-coated polystyrene beads were successively injected. Finally, the conductance variation was detected due to the presence of the beads bonded to the double antibody sandwich. Such a

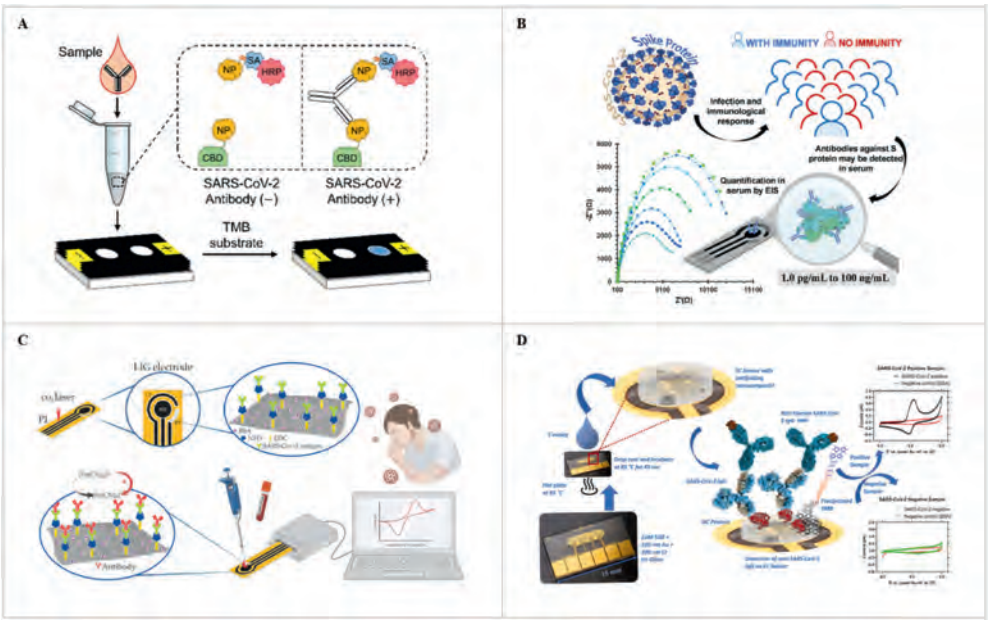

Figure 6 Schematic illustration showing electrodes based different materials coated used in SARS-CoV-2 Antibody Detection. (A) Schematic representation of Vertical-Flow Paper-Based Assays for SARS-CoV-2 Antibody Detection. Reprinted with the permission from Ref. 219. Copyright © 2021 American Chemical Society. (B) Schematic representation of an ultra-sensitive biosensor for antibodies against SARS-CoV-2. Reprinted with the permission from Ref. 221. Copyright © 2022 Elsevier. (C) Schematic representation of an electrochemical biosensor for SARS-CoV-2 biomarker antibodies. Reprinted with the permission from Ref. 223. Copyright © 2022 MDPI. (D) Schematic of the multiplexed EC sensor platform. Reprinted with the permission from Ref. 224. Copyright © 2023 Elsevier.

platform can reach nanoparticle resolution of few tens, which outperforms commercial ELISA kits with a LOD below 100 pg/mL. The system is perfectly suited to address a wide range of pathogens and diseases antibodies with clinically relevant concentrations for rapid immunoassays in a point of care setting[212].

4.1.3.4. Zika Virus. Zika Virus (ZIKV) is another member of the Flaviviridae family and is transmitted to humans by the same mosquito species as DENV[213]. In recent years, different biosensors have been developed for the detection of anti-ZIKV Abs. For example, the domain III of the envelope protein (EDIII) was commonly employed as recognition element for anti-ZIKV Abs detection. Based on dual antigens, ZIKV NS1 protein and EDIII, a novel biosensor was successfully developed to distinguish anti-ZIKV and DENV-specific Abs in blood and saliva[123]. Microwire sensors are versatile and highly durable to detect immune responses at the point of care. For example, a simple, robust capacitive microwire biosensor was developed by Geiss' group[214] using Zika or Chikungunya virus envelope antigen as recognition element. The proposed platform can detect as few as 10 antibody molecules in a small volume (10 molecules/30 μL) within only 10 min. More interestingly, both serological and molecular assays can provide a variety of information for the precise evaluation of Zika and Dengue infections. For example, Zika and dengue viral RNA detection down to a few nmol/L was carried out, and simultaneously, anti-ZIKV and anti-DENV Abs were detected in serum samples with LODs of 1.26 and 1.38 nmol/L, respectively. The results obtained in this dual assay format were comparable to those achieved in individual assays, proving its feasibility in clinical setting[215].

4.1.3.5. SARS-CoV-2. Since January 2020, COVID-19 has been spreading worldwide and has become a severe pandemic. Therefore, early detection of SARS-CoV-2 was extremely important for healthcare providers to make quick decisions and initiate effective control measures, which could significantly reduce mortality and economic loss[191]. Similarly, testing resultant antibodies is especially meaningful for vaccinated persons to monitor dynamic humoral response and further evaluate the effectiveness of vaccines[216]. Therefore, the research progress of biosensors for anti-SARS-CoV-2 Abs monitoring will be discussed.

The main structure of SARS-CoV-2 consists of a spike protein (S), an envelope protein (E), a membrane protein (M), and nucleocapsid (N) proteins. Specific biorecognition elements, such as S protein, angiotensin-converting enzyme 2 (ACE2), viral RNA sequence, aptamers and peptides, could specifically capture the anti-SARS-CoV-2 Abs (Fig. 6A)[217-220]. Recently, a series of studies have been carried out to develop biosensors for rapid and low-cost detection of anti-SARS-CoV-2 Abs. In an innovative and simple approach, carboxylated CNT with high conductivity were immobilized on a carbon electrode, which also increased the surface area for S protein adsorption, generating a stable layer able to capture the antibodies against SARS-CoV-2 in serum with high sensitivity (Fig. 6B). This novel electrochemical biosensor can be used post-infection or post-vaccination when circulating antibody concentrations are lower and cannot be detected by conventional ELISA methods[221]. Recently, an immunoassay platform for the determination of anti-SARS-CoV-2 Abs was fabricated by immobilizing the spike protein on gold clusters capped with cysteamine or mercaptoethanol. The platform could detect 0.03 fg/mL of the anti-SARS-CoV-2 Abs in synthetic media, spiked saliva or oropharyngeal swab samples. SWV measurements demonstrated approximately 10^9 times higher sensitivity than that of the lateral flow immunoassay method[138,222]. To meet the demand for effective diagnostic tools, biosensors with simple or no

Figure 7 Illustration of high-performance electrochemical biosensor devices for detection of SARS-CoV-2 antibodies. (A) Schematic representation of a paper-based electrochemical biosensor for detection of SARS-CoV-2 antibodies and antigen. Reprinted with the permission from Ref. 135. Copyright © 2021 Elsevier. (B) Schematic representation of an electrochemical biosensor for monitoring of SARS-CoV-2 biomarkers. Reprinted with the permission from Ref. 137. Copyright © 2021, The American Association for the Advancement of Science. (C) Schematic representation of an OFET biosensor for detection of SARS-CoV-2 antigen and antibodies. Reprinted with the permission from Ref. 232. Copyright © 2023 American Chemical Society.

sample pretreatment are required. Villarreal Carreño et al.[223] developed a disposable electrochemical biosensor containing graphene and an antigen specific to SARS-CoV-2 (Fig. 6C), which allowed for a fast differentiation of reactive and non-reactive patient serum samples within 3 min. Another electrochemical biosensor with pentaamine-modified graphene nanoflakes (prGOx) was also constructed. The prGOx coated working electrode could efficiently prevent biofouling for at least 9 weeks, making the assay more sensitive and selective in real biological samples (Fig. 6D). This sensor can also be utilized for rapid and quantitative detection of anti-SARS-CoV-2 antibodies using just 1.5 μL of plasma sample within 10 min with 100% sensitivity and specificity[224]. Sarkar et al.[225] introduced a novel electronic biosensing platform that utilizes synergistic enzyme-silver metallization on microelectrodes to enable sensitive, multiplexed detection of COVID-19 antibodies from ultra-low sample volume (<1 μL), eliminating the need for optical readouts while maintaining high specificity. The portable microchip system, coupled with a smartphone-compatible reader, successfully discriminated antibodies of infection versus vaccination, demonstrating potential as a low-cost, point-of-care alternative to conventional ELISA for simultaneous multi-biomarker analysis. Campuzano reported a multiplexed amperometric biosensor using magnetic beads functionalized with SARS-CoV-2 N and S proteins (including Delta/Omicron variants) for simultaneous detection of total antibodies and isotypes (IgG/IgM/IgA) at disposable 8-channel electrodes. The platform enables identification of vulnerable populations, immunity monitoring, vaccine efficacy evaluation, and variant-specific infection detection through sensitive immunoglobulin profiling[226]. Some other analytical platforms were constructed based on nickel hydroxide ($Ni(OH)_2$) NPs, ZnO nanorods, or magnetic nanoparticles, showing low LODs down to femtomolar concentrations[134,227-229].

To meet the demand for biodegradable, user-friendly, and less expensive biosensors, electrochemical paper-based analytical devices (ePAD) have attracted a broad interest. Herein, a paper-based electrochemical platform was used for anti-SARS-CoV-2 Abs detection, which is composed of three parts, a working ePAD on which the S protein receptor-binding domain (SP RBD) is immobilized, a counter ePAD, and a closing ePAD[135]. The developed COVID-19 ePAD for the serological detection of SARS-CoV-2 antibodies demonstrates potential applicability for qualitative screening of antibodies against SARS-CoV-2 (Fig. 7A). The detection of only anti-SARS-CoV-2 antibodies in clinical samples makes it difficult to provide comprehensive information for timely diagnosis. Therefore, the simultaneous analysis of different biomarkers, like nucleic acids, antigens, and antibodies, could help make more accurate decisions for virus monitoring and management. Subsequently, a smartphone-based three-in-one biosensor was prepared for co-detection of SARS-CoV-2 viral RNA, antigen, and antibody. The core sensing strategy was to adapt the 'sandwich' configuration for the three targets to different capture probes, each of which produced a current change after selective binding of the corresponding biomarker. Moreover, this sensor platform allowed for SARS-CoV-2 biomarkers determination at the femtomole level with good recovery results and had rapid electrochemical biosensing capability, information processing, and communication properties[230]. In response to the inconvenience of sample pre-treatment, a three-dimensional-printed, self-contained lab-on-a-chip (LOC) diagnosis platform was also proposed, which could be used to quantitatively detect SARS-CoV-2 RNA and host antibodies against this virus from unprocessed saliva samples. Multiplexed serology assays that target antibodies against several viral antigens are key to seroprevalence studies, which estimate the proportion of people in a population that has been infected, including asymptomatic infection, and

those immunized with vaccines. The multiplexed diagnostic tool integrates sample preparation and microfluidic chip. It is not only for POCT in healthcare and clinical setting, but also for estimating herd immunity and vaccine efficacy[231].

In recent years, organic field-effect transistors (OFETs) have been recognized as both high-performance electrochemical transducers and amplifiers to enhance biomolecular detection capabilities. The platform based on OFETs enabled the low-cost, label-free, and portable analysis of SARS-CoV-2 Abs, showing promise for use in the diagnosis and prognosis of COVID-19. For example, a platform based on OFET was constructed for the detection of SARS-CoV-2 Abs. This device, which could be remotely controlled by a mobile phone, showed ultralow detection limits in serum and saliva (Fig. 7B). Also, the reaction time between the antibody and the antigen (S protein) during incubation decreases as the voltage pulses on the gate electrode increase, so that the test can be completed within 5 min. Overall, OFET-based biosensors exhibit excellent specificity for antibody detection. The LODs of this biosensor for SARS-CoV-2 Abs can reach 1 fmol/L in aqueous solutions and 10 fmol/L in saliva and serum samples, which are much better than those of many existing electrochemical methods[137]. Furthermore, due to their potential flexibility and biodegradability, OFETs are prominent candidates for wearable and on-skin applications. An intrinsically soft and semiconducting triblock copolymer (TBC) film was adopted for the realization of stretchable OFET biological sensors, which could allow for low-cost fabrication and high sensitivity, as well as the development of applications for the simultaneous detection of SARS-CoV-2 antigen and antibodies (Fig. 7C). Moreover, the device demonstrates high sensitivity, with an LOD of 0.36 fg/mL for antibodies. At the same time, TBC used as an active layer can be stretched up to 90% with no crack formation of the film. In the future, this technology might be incorporated in garments or lab-on-a-mask systems for continuous health monitoring[232]. Furthermore, the integration of artificial intelligence (AI) offers improvements in electrochemical biosensor calibration and real-time data processing, facilitating the detection of molecular markers at ultra-low concentrations and enabling faster diagnostic timelines[233] Alves et al.[234] developed a laser-induced graphene immunosensor that achieves ultrasensitive anti-SARS-CoV-2 antibody detection (LOD: 0.032 μg/L) through electrochemically reduced graphene electrodes, overcoming cross-reactivity challenges from structural viral similarities and serum matrix effects. The introduction of an initial model of a neural network in the data analysis allowed to evaluate the positivity or negativity of the patients, making the diagnosis faster and more accessible. Currently. the post COVID monitoring is another important problem the world facing, electrochemical biosensors combined with 5G communication, machine learning (ML), internet-of-medical things (IoMT), and data clounding to transform diagnostic testing by offering high sensitivity, selectivity, and rapid response times and make new era toward hospital-in-chip (HOC) solutions[235].

4.2. Electrochemical biosensors for the detection of therapeutic antibodies

Unlike most drugs, therapeutic antibodies are more closely related to metabolic variability, and indirect predictors of patient metabolism, such as age, body mass, or genotype, are keys to ensure effective dosing. Hence, it is in great demand to develop highly sensitive and selective methods for the quantitative assay of antibody drugs. In this section, we provide a comprehensive overview of the electrochemical sensing strategies to meet the challenges associated with therapeutic antibody detection in the past ten years.

The majority of therapeutic antibodies currently available are developed based on the IgG framework. The high homology between hIgG and therapeutic antibodies in the constant region is one of the major factors affecting detection accuracy. To reduce false positives in therapeutic antibody detection, a series of affinity ligands (antigens, peptides, aptamers, etc.) utilized in electrochemical sensors are designed to target the fragment of antigen binding (Fab) region of the antibody, which exhibits distinct recognition specificity. For example, Zeng et al.[236] modified a HER2 mimotope (QLGPYELWELSH) by addition of seven amino-terminal (CGSGSGS) amino acids and then employed it to develop piezoimmunosensor or quartz crystal microbalance (QCM) assays for the detection of trastuzumab in human serum. Our group also introduced the amino acid sequence CGSGSGS as a linker into a peptide (WPRWLEN) targeting the Fab region of rituximab to obtain CN14 mimotope peptide. The dissociation constant (K_d) of CN14 was determined to be approximately 60 nmol/L through microscale thermophoresis (MST) experiments and the interaction mechanism was further explored by computer simulation. Additionally, this biosensor displayed satisfactory performance with a LOD of 35.26 ng/mL, showing great potential in TDM[77,81,159,237]. Then, a range of chimeric hairpin structures were designed for aptamers targeting bevacizumab (Fig. 8A), resulting in the identification of the aptamer with the highest affinity, H8 (8 complementary bps in the neck), which demonstrated a high affinity (K_d: 56.3 nmol/L). Thus, a one-step platform was constructed for the rapid determination of bevacizumab in a broad concentration range from 1 pmol/L to 1 μmol/L[145].

In particular, Ricci's group[96] (Fig. 8B) employed a 12-residue peptide excised from the epidermal growth factor receptor (EGFR) conjugated to PNAs for cetuximab detection. Furthermore, this group employed an EGFR antigen conjugated to DNA strands to develop more sophisticated recognition elements for the detection of cetuximab[141]. The aforementioned study reveals that novel recognition elements can effectively replace antigens for the precise recognition and detection of therapeutic antibodies. Building upon this, how to further improve the affinity and recognition efficiency of these recognition elements is one of the next avenues in the field of therapeutic antibody monitoring.

To address challenges such as low concentration of therapeutic antibodies and complexity of serum matrices, several signal amplification strategies have been proposed to improve the performance of electrochemical detection. Firstly, signal enhancement strategies based on nanomaterials were devised. Our group prepared a signal probe by adsorbing secondary antibodies onto gold nanoparticles (Fig. 8C), which markedly enhanced the impedance of the captured rituximab, resulting in an LOD of 35.26 ng/mL[81]. However, this immobilization approach of secondary antibodies on Au surface is typically uncontrollable and random, which will influence the reproducibility of the signal probe. Inspired by the highly efficient interactions of ordered structures in living organisms, we proposed a strategy for antibody orientation based on multifunctional DNA mediated spatially confined assembly (SCA). The multifunctional DNA containing a signaling molecule labeled on the head and a poly adenine tail,

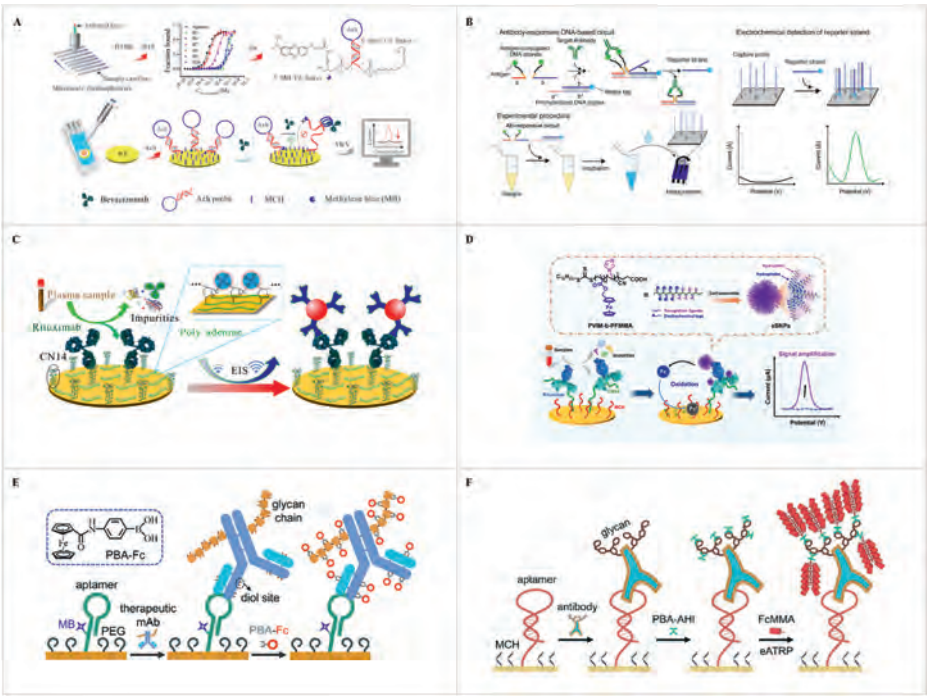

Figure 8 Illustration of peptide or aptamer as biorecognition elements for electrochemical biosensors to monitor therapeutic antibodies. (A) Schematic representation of a chimeric hairpin DNA aptamer for antibody detection. Reprinted with the permission from Ref. 145. Copyright © 2024 Royal Society of Chemical. (B) Schematic representation of DNA circuits for antibody detection. Reprinted with the permission from Ref. 96. Copyright © 2021 American Chemical Society. (C) Schematic representation of CD20 epitope mimetic peptide for rituximab detection. Reprinted with the permission from Ref. 81. Copyright © 2021 Elsevier. (D) Schematic representation of electroactive supramolecular nanoprobes for rituximab detection. Reprinted with the permission from Ref. 237. Copyright © 2024 Elsevier. (E) Schematic representation of amplification-free electrochemical detection of therapeutic mAbs. Reprinted with the permission from Ref. 144. Copyright © 2023 American Chemical Society. (F) Schematic representation of site-directed electrochemical grafting for amplified detection of antibody pharmaceuticals. Reprinted with the permission from Ref. 238. Copyright © 2024 American Chemical Society.

can not only direct the ordered immobilization of antibodies on gold nanoparticles, but can also enrich electrochemical signaling molecules. This makes the proposed biosensor appropriate for the accurate and sensitive analysis of rituximab in clinical plasma samples, with the obtained results being in good agreement with those of commercial ELISA[159]. To further enhance probe stability and adaptability in different environments, we prepared a novel block copolymer that simultaneously carries multiple recognition ligands (1-vinylimidazole, VIM) and redox tags (ferrocenylmethyl methacrylate, FMMA) to self-assemble into electroactive supramolecular nanoprobes (Fig. 8D), enabling sensitive and accurate monitoring of rituximab in plasma samples from non-Hodgkin's lymphoma patients without the need for secondary antibodies or complex modifications[237]. In addition, Hu's group[144] modified the diol sites on the glycan chains of therapeutic antibodies by boronate ester cross-linking with (4-ferrocenylacetamido) phenylboronic acid (PBA-Fc) for signal amplification (Fig. 8E). The peak current derived from MB-conjugated aptamers was employed as a reference signal for ratiometric detection, thereby enabling the amplification-free detection of bevacizumab. This group further developed a dual signal amplification strategy using site-specific electrochemical grafting of polymer chains for trastuzumab detection (Fig. 8F). The glycans of the antibody were modified with an alkyl halide initiator (AHI) *via* boronate ester cross-linking, after which ferrocene polymer chains were grafted from the antibody's glycan sites *via* electrochemically controlled atom transfer radical polymerization (eATRP), achieving a LOD of 71.5 pg/mL[238]. Finally, strategies based on DNA systems were systematically developed. Ricci's team combined the advantages of electrochemical detection with DNA-based circuits. In the presence of cetuximab, the release of a redox-tagged DNA strand was induced, which then hybridized with a DNA capture strand immobilized on the electrode, generating an electrochemical signal[96]. To further enhance electrochemical performance, Ricci's team replaced the redox reporter tags with glucose oxidase (GOx), thereby combining the programmability and multifunctionality of the DNA-based system with the sensitivity provided by enzyme-catalyzed amplification. The enzyme-linked DNA displacement (ELIDIS) platform enables the detection of five distinct antibodies, including BsAbs, through a straightforward alteration of the recognition elements conjugated to DNA strands[141].

While these strategies effectively achieve the high sensitivity requirements for antibody detection, they frequently involve several intricate steps, including multi-layer interface modifications, repeated washing protocols, enzyme-linked reactions, and DNA-based systems. Such complexity introduces significant challenges to the accuracy of electrochemical detection and real-time monitoring of antibodies in real samples. Consequently, there is a pressing need for the development of electrochemical sensing strategies with simplified operational procedures. Zeng et al.[158] provided a label-free and reagent-free peptide mimotope capacitive biosensor, where signal amplification was achieved by diluting the analysis buffer, leading to an optimized response time of less than 1 min. Sode et al.[94] designed an electrochemical sensor based on an anti-

idiotypic aptamer, capable of detecting bevacizumab within 30 min without the addition of free redox probes. Our group fabricated a simple electrochemical switch biosensor to detect bevacizumab in the concentration range from 1 pmol/L to 1 μmol/L in just one step[145]. Moreover, Bhardwaj et al.[93] manufactured an aptamer-modified polydimethylsiloxane-glass microfluidic chip for the on-line monitoring of ranibizumab using impedance-based electrochemical detection, which did not require sample preconcentration or preprocessing, and the detection time was reduced to 30 min. Despite the significant progress in the development of these sensor platforms, certain limitations remain, including the limited long-term stability of recognition elements and the potential for non-specific binding, which may adversely affect sensitivity and reproducibility in complex biological matrices. Nevertheless, with further refinement, these electrochemical sensors exhibit considerable potential for providing a rapid and effective alternative to conventional methods such as HPLC or HPLC−MS, particularly in clinical applications.

In summary, we have introduced novel strategies for the analysis of therapeutic antibodies. The characterization of the pharmacokinetics and pharmacodynamics (PK/PD) of therapeutic antibodies is essential not only for understanding their mechanisms of action, optimizing personalized medicine, and developing novel biosimilars, but also for ensuring the quality, safety, and efficacy of biomedicine. Besides, with the rapid advancement of therapeutic antibodies, the scope is being expanded to ADCs, BsAbs, BsADCs, nanobodies, etc. This expansion has resulted in increased detection demands and more complex applications, thereby giving rise to more challenges for electrochemical sensors, while focusing on more reliable medical care.

5. Conclusions and future perspectives

This review provides a comprehensive analysis of clinically relevant antibodies, beginning with an examination of the distinct roles of AAbs and therapeutic antibodies in disease diagnosis, monitoring, and prognosis. The detection of specific AAbs facilitates accurate diagnosis of autoimmune diseases, cancers, and viral infections. Moreover, monitoring therapeutic antibodies is essential for assessing their safety and efficacy. Following this, the review systematically categorizes the electrochemical sensors utilized in antibody detection, addressing recognition elements, signal transmission mechanisms, and device configurations, while also discussing the developmental trajectory and inherent challenges associated with these technologies. Finally, the review describes the specific applications of these electrochemical sensors across various disease contexts and examines the detection strategies for therapeutic antibody monitoring. Despite significant advancements in electrochemical sensors for clinically relevant antibodies, current implementations primarily focus on experimental stages, with commercial success and applications remaining relatively limited. Consequently, we discuss future directions and potential future avenues for the applications of electrochemical sensors specifically targeting clinical antibodies.

5.1. Future direction for the challenges

To achieve clinical adoption and commercial viability, future research must prioritize enhancing several critical performance parameters. (1) Developing robust and rapid signal amplification strategies for trace levels of antibodies in biological samples is paramount. Current amplification strategies, primarily based on nanomaterials, enzyme-catalyzed reactions and nucleic acid amplification, possess inherent limitations. For instance, while nanomaterials enhance ligand density and conductivity, they simultaneously exacerbate non-specific adsorption, leading to diminished S/N despite an overall signal increase. Meanwhile, challenges regarding nanomaterial heterogeneity and inconsistent ligand functionalization persist. Enzyme-catalyzed reactions are susceptible to activity fluctuations, introducing substantial uncertainties, particularly in multi-step reactions. Nucleic acid amplification suffers from prolonged reaction times and stringent temperature requirements. (2) Mitigating non-specific interference in complex matrices like serum is crucial, as interfering proteins vastly outnumber target antibodies in concentration and diversity, causing false positives/negatives. Although anti-fouling coatings (including PEG derivatives, zwitterionic polymers, peptides, polyadenylation, bovine serum albumin and biomimetic phospholipid layers) and self-cleaning surfaces (*e.g.*, photocatalytic coatings, electrochemical activation/regeneration) have been developed, future work should establish standardized protocols for evaluating antifouling efficacy by performing *in situ* electrochemical detection in clinically relevant samples rather than oversimplified buffers. (3) Improving stability, reproducibility and reliability requires durable recognition elements such as nanobodies, DNA origami structures, tetrahedral DNA nanostructures, cyclic peptide, combined with covalent or biomimetic anchoring and cross-linked polymer networks, can mitigate denaturation or leaching. Scalable micro-/nanofabrication techniques (*e.g.*, photolithography, ink-jet printing, laser writing) are also essential for ensuring batch consistency. Furthermore, dual-mode systems (*e.g.*, electrochemical/fluorescence) that self-calibrate across signal channels promise higher analytical confidence. (4) Advancing automation and integration represents an imperative trajectory for the next generation of wearable/implantable electrochemical sensors. Significant progress in miniaturization and multifunctional integration technologies is poised to dramatically enhance sensor portability, user-friendliness, and accessibility, enabling robust POCT across diverse clinical and potentially even home settings. The incorporation of sophisticated microfluidic systems and LOC architectures has been instrumental in developing compact, portable diagnostic platforms. However, translating these advancements faces critical technical hurdles. Key among these are concerns regarding the long-term stability and biofouling resistance of biorecognition elements (*e.g.*, enzymes, antibodies, aptamers) within complex biological fluids, which can degrade sensor performance and reliability over extended periods. Furthermore, the scalability and cost-effective mass manufacturing of intricate microfluidic devices present significant challenges that must be addressed to achieve widespread clinical adoption. Concurrently, the integration of AI and ML algorithms is revolutionizing data processing. These powerful tools enable the automatic analysis of intricate electrochemical signals, sophisticated pattern recognition, effective background subtraction, and automated result interpretation, thereby substantially improving analytical accuracy, reliability, and enabling intelligent, real-time data management. Looking forward, realizing the full translational potential of these integrated, intelligent systems requires addressing not only technical limitations but also navigating complex regulatory pathways and carefully considering emerging ethical challenges. These ethical considerations encompass data privacy and security vulnerabilities inherent in wirelessly transmitted sensitive health information, potential biases embedded within AI algorithms, and societal implications regarding equitable

access to these advanced diagnostic technologies. Sustained research and development investment in these key directions hold the promise for electrochemical sensors targeting clinically relevant antibodies to overcome current bottlenecks and achieve a transformative leap from laboratory proof-of-concept to reliable, efficient, and widely adopted clinical diagnostic tools, ultimately providing robust technological support for precise disease diagnosis, therapy monitoring, and personalized medicine.

5.2. Future avenues for the applications

5.2.1. High-throughput screening and discovery of autoantibody-based biomarkers

Given the limited diagnostic capacity of individual AAbs, a combination of multiple AAbs is essential to enhance overall sensitivity and accuracy. The protein array technology plays a pivotal role in investigating protein−protein interactions and identifying antigenic targets of serum AAbs across various autoimmune disorders. The advantages of screening immune responses to a large array of proteins using high-throughput techniques not only improve diagnostic accuracy but also facilitate the identification of autoantibody signatures that may delineate disease subgroups and enable early diagnosis[239]. Moreover, these techniques can be employed to analyze vaccine trials[240]. Electrochemical sensors possess the advantages of miniaturization and high throughput, and can also be used as a promising technology for detecting protein−protein interactions. When combined with protein array technology, these sensors hold the potential to enhance the screening and discovery of novel autoantibodies, ultimately improving the accuracy of disease diagnosis.

5.2.2. Detection of antibody−drug conjugates, bispecific antibodies and their conjugates6

ADCs, BsAbs, and their conjugates are representative of the newer generation of therapeutic antibody formats, with several approved drugs and numerous candidates currently in clinical development. ADCs are immunoconjugates comprising a monoclonal antibody tethered to a cytotoxic drug (known as the payload) *via* a chemical linker, which are designed to selectively deliver the payload directly to the target cancer cells through the antibody[241]. BsAbs are engineered proteins that bind to two different types of antigens or two different epitopes on the same antigen, which enable novel mechanisms of action or therapeutic applications that cannot be achieved using conventional IgG-based mAbs[9]. The structural complexity and heterogeneity of BsAbs and ADCs complicate their analysis and detection, which makes it challenging to achieve their precise quantification using a single target antigen or recognition element. Because of the high failure rate during drug development, it is necessary to track their therapeutic and toxicological effects. Beyond serum concentration monitoring, understanding where ADCs distribute (either within cells or throughout the body) after administration and how they accumulate in target tissues is crucial for elucidating their mechanisms of action[55]. This information can provide insights into ADC interactions with cells, cellular uptake, and elimination, which may not be obtained *via* conventional *in vitro* analytical tools. Therefore, implantable electrochemical sensors may serve as novel analytical devices for therapeutic antibody distribution *in vivo*.

5.2.3. Evaluation of immunogenicity

The administration of biopharmaceuticals inevitably triggers an immune response, which may result in the generation of anti-drug antibodies (ADAs) to clear the drug, a phenomenon known as immunogenicity. ADAs are classified into non-neutralizing antibodies (non-NAbs) and neutralizing antibodies (NAbs). Non-NAbs bind to biopharmaceuticals without disrupting their interaction with target sites. Conversely, NAbs could compete for binding sites and weaken therapeutic efficacy. For instance, ADAs produced against epoetin-α (EPO) can neutralize endogenous EPO, inducing pure red cell aplasia (PRCA). Similarly, NAbs elicited by exogenous IFN-β may form immune complexes with endogenous IFN-β and activate the complement system. NAbs associated with coagulation factor VII (FVII) can exacerbate pre-existing hemorrhagic conditions. Consequently, the immunogenicity of therapeutic antibodies has emerged as a widespread concern for clinicians, pharmaceutical manufacturers, and supervision departments. To accurately evaluate the immunogenicity of biopharmaceuticals, it is essential to develop robust analytical methods for the effective evaluation of ADAs in both preclinical and clinical contexts. Therefore, electrochemical biosensors are anticipated to be a crucial area for the monitoring of ADAs.

In conclusion, the accelerated progress of electrochemical biosensors has positioned them for extensive application to antibody detection in clinical settings. The integration of multidisciplinary knowledge provides the opportunity for these devices to interface with AI, biology, and soft electronics, which is likely to facilitate their widespread adoption in hospitals and other healthcare institutions.

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Author contributions

Zheng Zhao: Writing-original draft, Data curation. Zhiwei Chen: Writing-original draft, Data curation. Jacques Crommen: Validation, Writing-review & editing. Shengfeng Huang: Conceptualization, Supervision, Writing-review & editing. Qiqin Wang: Conceptualization, Supervision, Funding acquisition, Writing-review & editing. Zhengjin Jiang: Conceptualization, Supervision, Funding acquisition, Writing-review & editing. All authors have approved the final review and the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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