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REVIEW

Electrochemical biosensors for point-of-care testing

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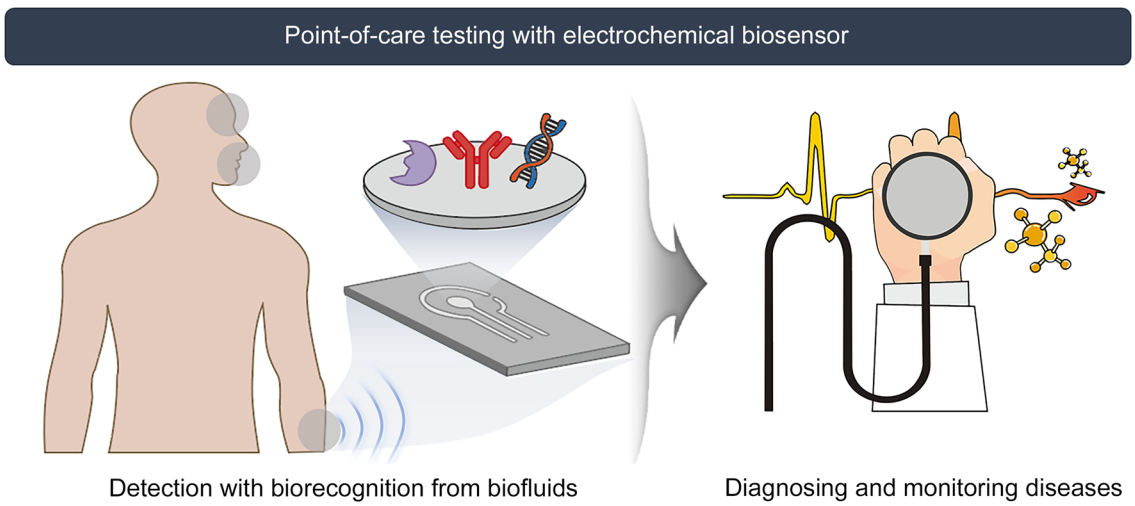
Abstract

Point-of-care testing (POCT) is the practice of diagnosing and monitoring diseases where the patient is located, as opposed to traditional treatment conducted solely in a medical laboratory or other clinical setting. POCT has been less common in the recent past due to a lack of portable medical devices capable of facilitating effective medical testing. However, recent growth has occurred in this field due to advances in diagnostic technologies, device miniaturization, and progress in wearable electronics. Among these developments, electrochemical sensors have attracted interest in the POCT field due to their high sensitivity, compact size, and affordability. They are used in various applications, from disease diagnosis to health status monitoring. In this paper we explore recent advancements in electrochemical sensors, the methods of fabricating them, and the various types of sensing mechanisms that can be used. Furthermore, we delve into methods for immobilizing specific biorecognition elements, including enzymes, antibodies, and aptamers, onto electrode surfaces and how these sensors are used in real-world POCT settings.

Graphic abstract



Keywords Point-of-care testing (POCT) · Electrochemical sensor · Enzyme · Antibody · Health care

Introduction

Point-of-care testing (POCT) refers to medical testing and diagnostic procedures performed in a patient's home or

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at other locations outside traditional medical facilities [1]. Unlike conventional diagnostic tests performed in laboratory settings, POCT can provide rapid and real-time results, making it critical for effective patient diagnosis and treatment [2]. Point-of-care (POC) devices must produce reliable results quickly and be highly portable to enable patient-friendly diagnosis [1–3]. Electrochemical sensors have strong potential for integration into POCT systems because they offer high sensitivity, accuracy, specificity, low detection limits, can be miniaturized, are cost-effective, and are easy for users to operate [4, 5].

The electrochemical sensor system comprises three primary components: a biorecognition function, an electrochemical transducer, and a signal conversion module [6]. Electrical or electrochemical reactions occur at the interface of an electrode and a sample solution; here, the biorecognition element and target analyte are combined on the working electrode surface [7]. Electrochemical signal transducers track the electrical signals produced by this reaction, including the electrical current or potentials produced, for measurement or detection. Typically, carbon and metallic electrodes are commonly used as transducers, and they can be modified with biorecognition materials such as biomolecules or biocatalysts to enhance the electrochemical sensitivity and specificity of the sensors. Finally, signal acquisition modules process data received from the transducer and display the results. A schematic of this procedure is shown in Fig. 1.

Electrochemical sensors implemented in POC devices are used to analyze various biological fluids [6, 8]. A biofluid refers to one of various biological fluids generated by the human body. Biofluids provide valuable insight into the physiological responses and activities of the body, making them crucial for various applications including diagnostics, monitoring, and research. Consequently, they may be valuable for POCT. Blood, one of the main biofluids used in medical testing, contains a wealth of information because its components, including cells, plasma, and platelets, perform different physiological functions. However, blood sampling for electrochemical sensing involves invasive procedures such as finger-pricking or venipuncture, which can cause discomfort for users of POC devices. Accordingly, recent research has focused on the detection of biomarkers using alternative biofluids that can be sampled noninvasively. For example, interstitial fluid, which is found between cells and blood vessels in many tissues, is a medium used for the transport of substances such as nutrients and waste products. It has a chemical composition similar to that of plasma because it is produced as the plasma leaks out of the circulatory system. Interstitial fluid is a promising sample for noninvasive health monitoring since it can be collected transdermally using ultrasound and reverse iontophoresis. Sweat is another biofluid that has been widely used in recent wearable biosensors. Sweat, which consists of ions, amino acids, and proteins, controls body temperature and removes waste from the body. Since sweat can be easily collected during physical exercise, iontophoresis, or exposure to high temperatures, it enables noninvasive monitoring of a patient's physiological condition. Saliva is a biofluid produced in the oral cavity that aids digestion and maintains oral health. It can also be collected noninvasively and is rapidly regenerated, which allows for the swift detection of changes in physiological state. Furthermore, saliva contains many biological components and is therefore an attractive choice for electrochemical sensors. Tears are another alternative biofluid that contains ions, sugars, and proteins. However, extracting information from tears via bioelectronic sensors presents many challenges and remains at a low state of technological development. Overall, biofluids offer the unique advantage of being able to be collected noninvasively, and therefore they are widely used in POC devices. Moreover, various specialized analysis and research techniques have been developed based on their respective characteristics and compositions.

Electrochemical sensors use various measurement methods including voltammetry, amperometry, impedimentary, and potentiometry to detect the presence and concentration of various chemical substances [8, 9]. Voltammetry involves measuring the electrical current while varying electrode potential and includes techniques such as differential pulse voltammetry, cyclic voltammetry (CV), linear sweep voltammetry, and square wave voltammetry (SWV). This measurement method is commonly used in biosensors due to its affordability, high sensitivity, and high precision. Amperometric sensors detect electrochemical reactions by measuring the current. Sensitivity can be determined by comparing the current measured at various analyte concentrations. Impedimetric measurement evaluates the substance concentration and other characteristics via measurement of the electrical impedance generated in proportion to an analyte's activity on the electrode surface. One of these methods, electrochemical impedance spectroscopy (EIS), is an important experimental technique employed in the design of electrochemical sensors. EIS primarily provides information regarding the rate and mechanisms of electrochemical reactions, electrode surface properties, and electrolyte electrical characteristics. Finally, potentiometry is a technique used to determine the concentration of a chemical substance by measuring the potential change caused by ion exchange or charge transfer on the electrode surface. This method is commonly used in pH sensors and ion-selective electrodes. Overall, electrochemical sensors combine and adapt these diverse measurement methods to meet the needs of specific applications.

Among the various types of electrochemical biosensors, there are two commonly used sensors with distinct characteristics used for biological recognition: enzyme- and antibody-based sensors [7]. Enzymatic sensors use enzyme

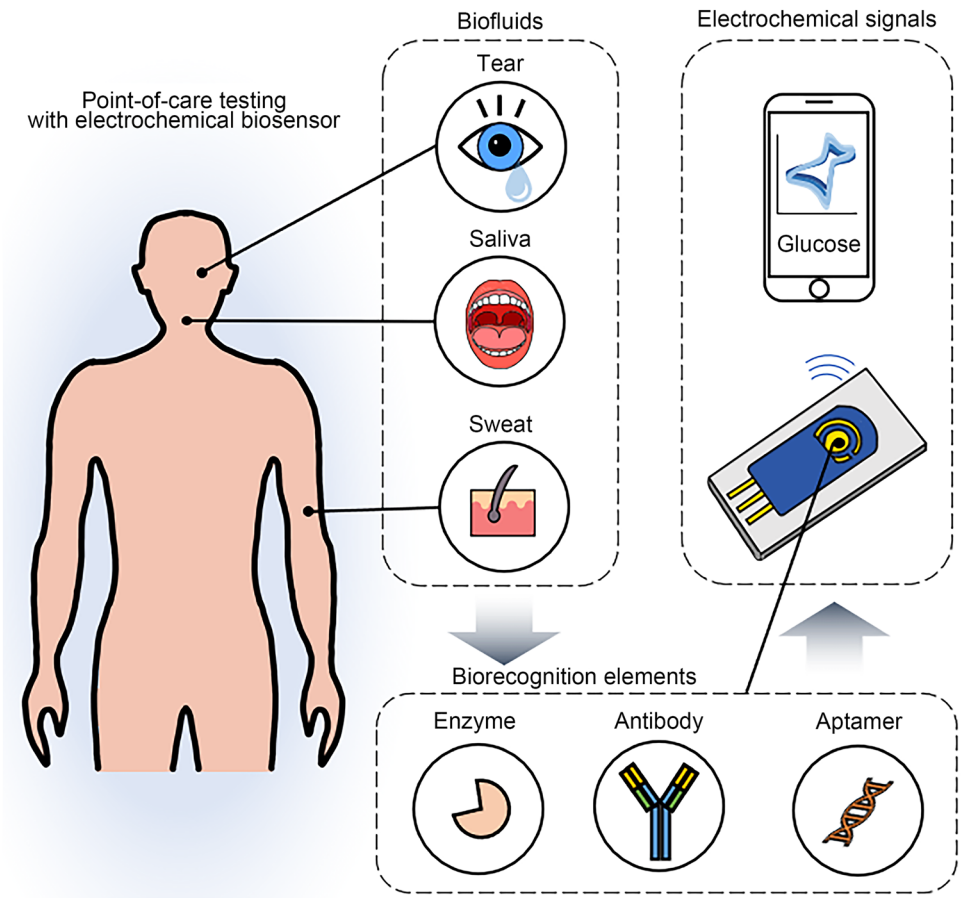

Fig. 1 Schematic representation of electrochemical systems for the detection of various analytes in biofluids using bioreceptors

activity to detect the presence or concentration of specific substances. In general, enzymes interact with specific substances, and this action induces an electrochemical reaction that is measured by changes in current or voltage on the electrode surface. It is often used to measure the concentration of specific substances, such as glucose sensors which measure glucose concentration. Another electrochemical sensor design uses antibodies. Electrochemical sensors using antibodies measure biosignals generated by the specific binding of antibodies. Antibody-based electrochemical sensors are employed in diverse fields, including cancer diagnostics, biomarker detection, pathogen detection, toxic substance detection, and in biological research. Enzyme- and antibody-based electrochemical sensors are both highly sensitive and selective, and act as essential analytical tools for various applications. These sensors enable rapid result acquisition and real-time monitoring, making them valuable for medical diagnostics, environmental monitoring, food safety assessment, and for fundamental research throughout the biosciences. This paper provides a detailed introduction to enzyme- and antibody-based electrochemical sensors, their electrode immobilization techniques, and their applications for POC devices.

Enzyme-based electrochemical biosensors

Enzyme-based electrodes for biosensors have the advantage of using the unique properties of enzymes to electrochemically detect target substances. First, enzymes exhibit high sensitivity and specificity because they specifically interact with target molecules or chemicals, thereby permitting the detection and quantification of targets at low concentrations. In addition, certain enzymes possess a high degree of selectivity, so they react only with specific target substances. This selectivity ensures the accurate detection of desired target substances while avoiding interference from the presence of other chemical compounds. Depending on the method of electrode fabrication, enzymes can be reusable, thereby enhancing cost-effectiveness. Enzyme-based sensors also offer short response time, enabling real-time monitoring and control. In the following sections, we explore different enzyme-based electrode fabrication methods and discuss illustrative example use cases.

Fabrication process for immobilizing enzymes on the electrode surface

Enzyme immobilization, which involves physically or chemically attaching enzymes to the electrode surface, is a crucial step for effective biosensor production. This process enhances sensor operational and storage stability and facilitates high sensor sensitivity, selectivity, and reproducibility, while also permitting short response time. Immobilized enzymes must maintain their structure and function to preserve reactivity following immobilization. The choice of the method of immobilization can affect enzyme activity and the stability of resulting enzyme-based sensors. Moreover, since factors such as biosensor analytic performance, reproducibility, and operational lifespan are affected by the immobilization process, many studies have focused on the development of successful immobilization methods. As illustrated in Fig. 2a, common immobilization methods include adsorption, covalent bonding, cross-linking, and entrapment; these methods differ with respect to how interactions between enzymes and electrodes or among enzymes are allowed to occur. Table 1 summarizes the mechanisms, advantages, and

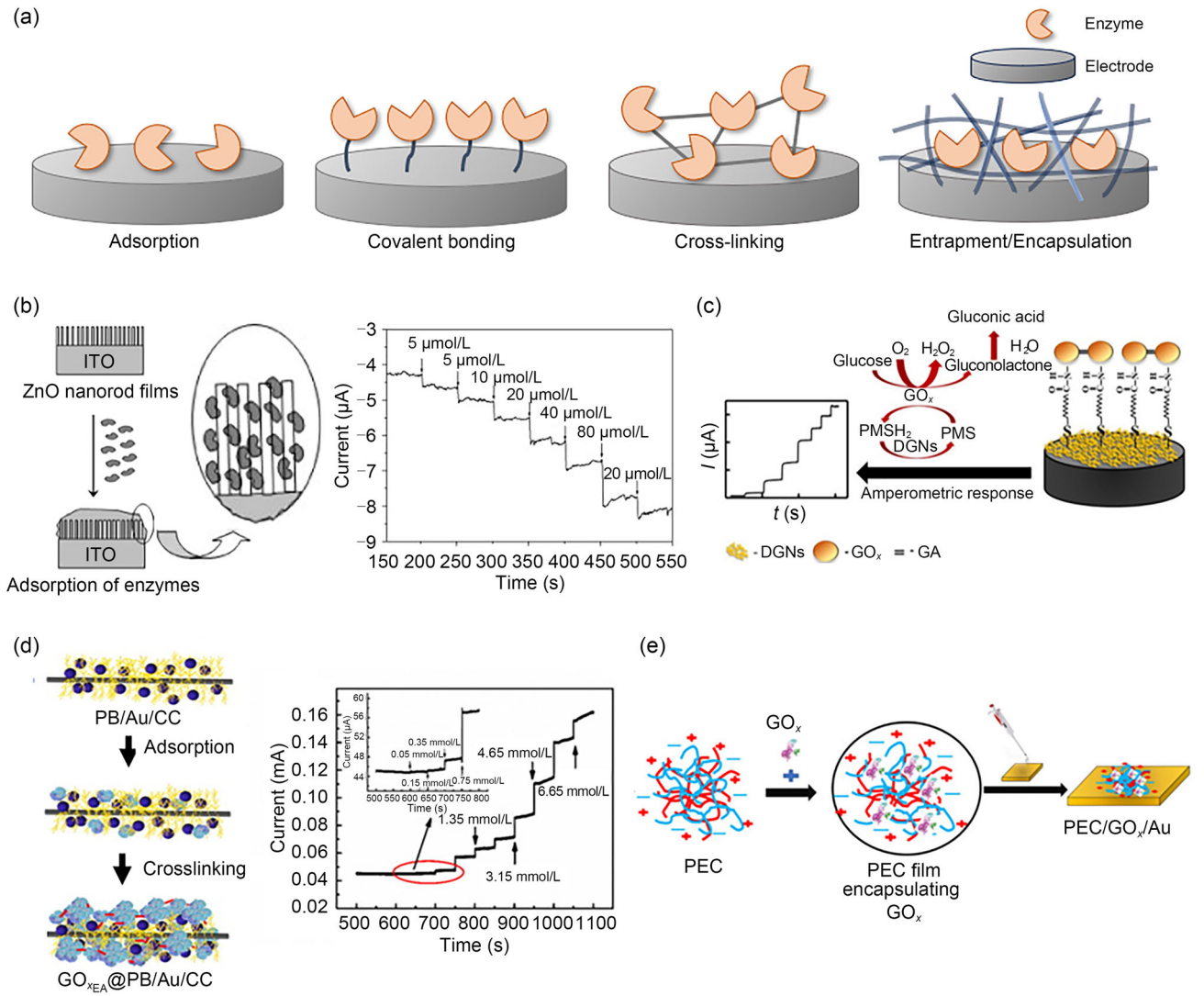

Fig. 2 **a** Schematic representation of methods used to immobilize enzymes on electrodes. **b** Enzymes immobilized on aligned ZnO nanorod film electrodes using the adsorption method (reproduced from Ref. [11], Copyright 2009, with permission from Elsevier). **c** Modification of the graphite rod (GR) electrode by dendritic gold nanostructures (DGNs) and immobilization of glucose oxidase (GO_x) for electrochemical determination of glucose (reproduced from Ref. [14], Copyright 2022, with permission from the authors, licensed under CC BY 4.0). **d** Schematic depicting the formation process of the GO_{xEA}@PB/Au/CC electrode (reproduced from Ref. [22], Copyright 2021, with permission from Elsevier). **e** A fabrication process for a glucose biosensor based on glucose oxidase encapsulation in a chitosan–kappa–carrageenan polyelectrolyte complex (reproduced from Ref. [29], Copyright 2018, with permission from Elsevier)

Table 1 Advantages and disadvantages of various enzyme immobilization methods

Enzyme immobilization method	Mechanism	Advantages	Disadvantages
Adsorption	Weak interactions between the electrode and the enzyme	Simple and intuitive Low cost Limited loss of enzyme activity	Enzyme detachment due to weak binding
Covalent bonding	Formation of covalent bonds between functional groups of the enzyme and the support matrix	High enzyme stability Preventing enzyme leakage	Risk of enzyme denaturation due to chemical modification Requires large volumes of bioreagent
Cross-linking	Cross-linkage formation between enzyme molecules via covalent bonding	Simple Low enzyme loss due to strong chemical bonds between enzyme molecules	High enzyme deformation that can lead to loss of activity
Entrapment/Encapsulation	Confinement of enzymes within a polymeric network	High enzyme stability Low enzyme leakage Minimal enzyme deformation	Diffusion barrier between electrodes and enzymes

disadvantages of several common enzyme immobilization methods.

Adsorption

Adsorption is a straightforward and intuitive approach for physically immobilizing enzymes on a fixed surface. This approach involves attaching enzymes to a surface by taking advantage of weak interactions between the electrode and the enzyme such as van der Waals forces, electrostatic interactions, or hydrophobic interactions. This process typically involves dissolving enzymes in a solution and incubating the solid support in the enzyme solution at an appropriate temperature for a certain period to maintain enzyme activity. Although adsorption is susceptible to enzyme detachment when temperature, pH, or ionic strength conditions change, thereby weakening bond strength, it remains widely used because of its simplicity and cost-effectiveness [10]. Liu et al. successfully immobilized glucose oxidase using the adsorption method onto aligned zinc oxide (ZnO) nanorod films grown on indium tin oxide (ITO) substrates, as shown in Fig. 2b [11]. ZnO possesses a high isoelectric point, which allows it to interact electrostatically with acidic proteins such as glucose oxidase during immobilization. Enzymes fixed on the ZnO nanorod surface demonstrated exceptional stability and high catalytic activity for glucose detection.

Covalent bonding

The covalent bonding method is a widely used enzyme immobilization method that involves the formation of a covalent bond between an enzymatic functional group and the support matrix. This method enhances enzyme stability, and the bonds that form are strong, preventing enzyme leakage. Moreover, it is essential that unnecessary functional groups that interfere with enzyme activity are not involved in the bonding process, and catalytic activity may decrease if amino acids that are essential for enzyme activity become part of the bonding [12]. Typical functional groups used for covalent bonding include amino, carboxylic, phenolic, sulfhydryl, thiol, imidazole, indole, and hydroxyl groups [13]. The process of enzyme binding to the electrode typically involves two steps. First, the electrode is treated with molecules such as glutaraldehyde or carbodiimide, which act as linkers. Next, these linker molecules are immobilized on the electrode surface, where they form a self-assembled monolayer (SAM) that serves as a bridge between the surface of the electrode and the enzyme via covalent bonding. One drawback of this method is that when enzymes are covalently attached, there is a risk of chemical modification and enzyme denaturation. In addition, this approach often requires large volumes of bioreagents, even though the actual amount of enzyme immobilized is relatively small. However, various linkers can be employed on a wide variety of surfaces including inorganic materials, polymers, and membranes [14–20]. The specific

immobilization protocols used can vary, and can involve placing enzymes directly on the transducer surface or depositing enzymes in a thin film on the transducer. Recently, a glucose biosensor was developed using a graphite rod modified with Au nanostructures, glucose oxidase (GO_x), and phenazine methosulfate (PMS) [14]. After immobilizing the enzyme in various ways, the authors found that the approach depicted in Fig. 2c, in which GO_x is covalently attached to an SAM and subsequently cross-linked, generated a significantly higher maximum current than other methods. Furthermore, this finding demonstrated that the detectable range for glucose measurement extended from 0.1 to 10 mmol/L, with a minimum detectable concentration of 0.019 mmol/L.

Cross-linking

Cross-linking is a carrier-less method that allows enzyme immobilization via the formation of cross-linkages through covalent bonds between enzyme molecules. Cross-linking agents such as glutaraldehyde create enzyme aggregates known as cross-linking enzyme aggregates (CLEAs) [21]. The use of cross-linking to immobilize enzymes is attractive due to its simplicity and the formation of strong chemical bonds between enzyme molecules, which results in minimal enzyme loss. However, using glutaraldehyde for cross-linking can cause significant enzyme modification and the subsequent loss of enzyme activity [9]. Therefore, inert proteins such as gelatin and bovine serum albumin (BSA) can be incorporated to mitigate the significant alteration of enzymes during the immobilization process. In one study, Yan et al. developed an electrochemical sensor for glucose sensing using Prussian blue, a widely used electrocatalyst, and glutaraldehyde to form glucose oxidase aggregates (process illustrated in Fig. 2d) [22]. The CLEAs generated in this study enhanced performance by enabling the immobilization of a large quantity of enzymes on the electrode.

Entrapment

Enzyme immobilization via entrapment involves confining the enzyme within a polymeric network rather than directly attaching it to the support surface. Entrapment generally proceeds in two steps: initially, the enzyme is blended with a solution containing many monomers, which is subsequently solidified via polymerization [23–29]. This approach effectively restricts enzyme diffusion while allowing movement of both reactants and products. It enhances enzyme stability, prevents enzyme leakage, and minimizes biological alteration during the immobilization process, thereby reducing the risk of loss of enzyme activity. As shown in Fig. 2e, Rassas et al. designed a voltammetric glucose sensor by immobilizing glucose oxidase within a chitosan–kappa–carrageenan polyelectrolyte complex (PEC) [29]. This biosensor, which employed SWV, demonstrated sensitivity to glucose over a wide linear range (i.e., 5 μmol/L to 7 mmol/L) with a low detection limit.

Applications of enzyme-based electrochemical biosensors for POC devices

Glucose sensors

At present, diabetes—a chronic metabolic disorder—is one of the most significant global health issues. Consequently, research on glucose detection technologies has become highly important for managing and preventing diabetes. In healthy people, fasting blood glucose levels typically range from 3.9 to 5.6 mmol/L [30]. However, the levels of patients with diabetes exceed the normal range, and it is therefore crucial to monitor glucose concentrations to assess patient condition. Traditional devices provide only point sample information, but the adoption of continuous glucose monitoring technology has provided more comprehensive data on the glucose fluctuations of diabetic patients. In addition, there is rapidly growing demand for POC technologies that enable diabetic patients to measure glucose values and obtain medical test results themselves, thereby improving their quality of life. Enzymatic biosensors based on glucose oxidase are widely used in various detection technologies employed by POC devices. Enzymatic glucose sensors have been highly regarded due to their simplicity, high sensitivity, selectivity, broad linear range, and low detection limit. Furthermore, research into how to integrate these sensors into devices, such as drug delivery systems, remains ongoing. The goal of such research is to develop comprehensive and integrated systems for diabetes management.

Commercially available glucose sensors that rely on invasive approaches such as finger pricks not only hinder patient compliance but also pose risks associated with skin damage and bacterial infection. Extensive research is being conducted on noninvasive glucose monitoring technologies. For example, other biofluids, including sweat, saliva, and tears, are potential candidate samples that can be used for glucose monitoring [31–37]. For example, the use of noninvasive sweat-based glucose sensors to estimate blood glucose levels is a promising approach. In one study, Lee et al. designed a wearable and disposable sweat-based glucose device, shown in Fig. 3a [33]. During the construction of this device, they used electrodeposition to create porous gold electrodes and then immobilized enzymes by drop-casting a mixture of chitosan/graphene solution, GO_x, and BSA. Real-time correction for glucose concentration was performed by measuring pH, temperature, and humidity. They established a feedback-controlled transdermal drug delivery module to facilitate drug release in response to glucose levels. This innovative closed-loop system was found to be able to monitor

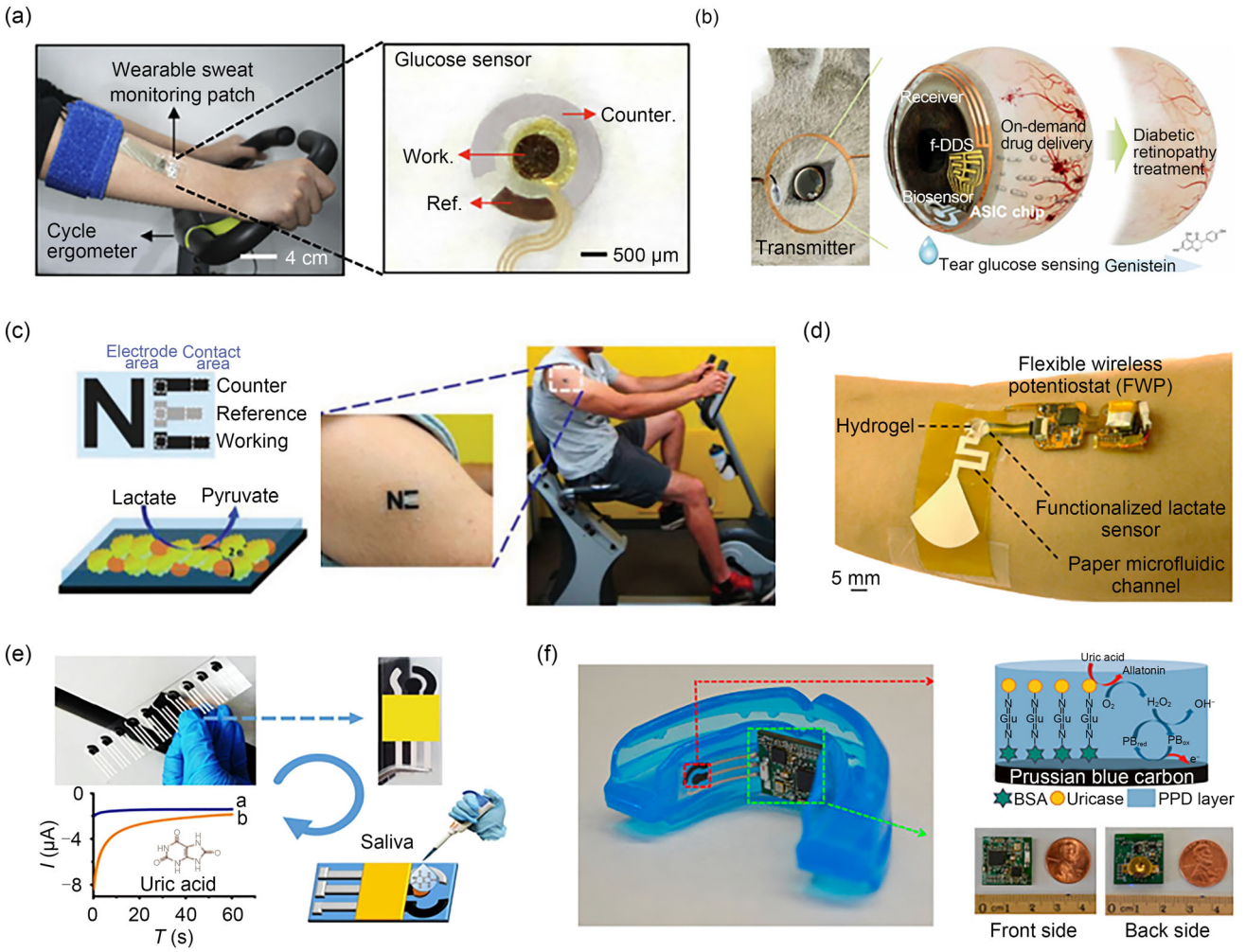

Fig. 3 **a** A wearable and disposable sweat glucose monitoring device, featuring a multistage transdermal drug delivery module (reproduced from Ref. [33], Copyright 2017, with permission from the authors, licensed under CC BY-NC 4.0). **b** Schematic representation of a remotely controllable smart contact lens for noninvasive glucose monitoring (reproduced from Ref. [34], Copyright 2020, with permission from the authors, licensed under CC BY-NC 4.0). **c** An "NE" tattoo biosensor for electrochemical epidermal monitoring of lactate applied to a male volunteer's deltoid (reproduced from Ref. [45], Copyright 2013, with permission from the American Chemical Society). **d** An integrated osmotic wearable system for sensing lactate in sweat. This apparatus consists of an osmotic hydrogel, a paper microfluidic channel, a functionalized lactate sensor, and a flexible wireless potentiostat (reproduced from Ref. [46], Copyright 2022, with permission from the American Chemical Society). **e** An electrochemical biosensor using a screen-printed electrode modified with multiwall carbon nanotubes and immobilized with uricase for the detection of uric acid (UA) in saliva (reproduced from Ref. [57], Copyright 2020, with permission from Springer-Verlag GmbH Germany, part of Springer Nature). **f** Description of a mouthguard biosensor featuring a wireless amperometric circuit board and a chemically modified printed Prussian blue carbon working electrode with uricase for sensing UA (reproduced from Ref. [60], Copyright 2015, with permission from Elsevier)

diabetes mellitus noninvasively by measuring sweat, thereby offering a novel way to manage diabetes. In another study, the same group developed a patch based on graphene to monitor glucose levels in sweat and to deliver metformin to lower blood glucose levels [31]. Like sweat, tears are an attractive biofluid for noninvasive monitoring due to their composition, which includes not only plenty of proteins designed to maintain healthy eye surfaces but also salts, metabolites, and immunoglobulins [38]. Keum et al. created a functional contact lens that can be controlled remotely for noninvasive glucose monitoring and precise administration of medications for treating diabetic retinopathy, as illustrated in Fig. 3b [34]. For this device, the authors immobilized glucose oxidase along with BSA, poly(vinyl alcohol), and chitosan on a Pt electrode through covalent bonding and cross-linking between the enzyme and the substrate. Moreover, when the glucose concentration was increased from 5 to 50 mg/dL, the measured current was found to increase from 0.4 to 3.1 μA, demonstrating a suitable current change for remote glucose monitoring. Further in vivo experiments were conducted

by placing this device in the eyes of diabetic rabbits. This experiment demonstrated that the device could measure tear glucose values that closely matched the blood glucose level profiles quantified using a commercial glucose sensor. Overall, this innovative technology holds promise for noninvasive glucose level detection and treatment of diabetic retinopathy.

Lactate acid sensors

Lactate is an essential metabolite produced by anaerobic metabolic processes in muscle cells when oxygen levels are low. The accumulation of lactate in the muscles during exercise can lead to muscle pain, making it an important biomarker for monitoring and assessing muscle fatigue [39, 40]. While healthy individuals at rest typically have blood lactate concentrations ranging from 0.5 to 2 mmol/L, during exercise this can increase to approximately 12 mmol/L [41–43]. Moreover, since most of the lactate within muscle cells is excreted through sweat, the sweat lactate concentration is generally 2–10 times higher than the blood lactate concentration, which ranges from 2 to 115 mmol/L [44]. In addition, given the growing interest in noninvasive detection methods, many lactate electrochemical sensors are being developed that use sweat as a sample. Jia et al. created a skin-worn enzymatic biosensor capable of sensing lactate levels in perspiration [45]. In that paper, a lactate sensor was created by dropping-casting a mixture of lactate oxidase (LO_x) and BSA stabilizer oxidizer on a working electrode printed with multiwalled carbon nanotubes (MWCNTs) and Tetrathiafulvalene (TTF). In vitro experiments showed that this tattoo sensor displayed a highly linear response within a lactate concentration range of 1–20 mmol/L. Furthermore, 10 healthy volunteers were then recruited to monitor the real-time response of the tattoo sensor during periods of cycling exercise (Fig. 3c). Taken together, these results indicated that compared to a control group that lacked the additional enzyme, the LO_x-functionalized tattoo biosensor demonstrated excellent biocatalytic ability for oxidizing lactate in sweat. It was also observed that epidermal lactate profiles closely tracked exercise intensity with little or no lag. Therefore, the LO_x-functionalized biosensor may be a valuable tool for detecting lactate levels associated with exercise intensity, and may thereby help to facilitate the evaluation of individual fitness and physical activity levels.

Some sweat-based lactate biosensors include integrated sweat stimulation to extract sweat and measure lactate concentration, even during resting conditions, which are typical activity conditions for many people [46, 47]. For example, Saha et al. developed an electrochemical sensing platform combined with osmotic sweat extraction to continuously monitor sweat lactate, as shown in Fig. 3d [46]. They used hydrogels with a higher osmotic strength value than sweat, which permitted sweat extraction without the need for components that require electrical power. Moreover, persistent evaporation occurring at the endpoint of a paper-based channel has been found to facilitate the measurement of lactate in fresh sweat. In this study, screen-printed electrodes (SPEs) with immobilized LO_x were used on the sweat collection site of the skin to enable dynamic lactate level assessment. In addition to osmotic pumping, iontophoresis is widely used to extract sweat for lactate sensing [47–49]. Xuan et al. developed an epidermal patch that can sense lactate levels based on sweat produced in response to iontophoresis stimulation, thereby offering significant potential for applications in health care [48].

Uric acid sensors

Uric acid (UA) is the ultimate product of the purine metabolic pathway within the human body. The amount of UA in the bloodstream of healthy individuals typically ranges from 240 to 520 μmol/L [50]. Exceeding this range can lead to serious diseases such as gout [51], hyperuricemia [52], and Lesch–Nyhan syndrome [53]. Elevated UA levels are also associated with the risk of obesity [54], cardiovascular disease [55], and kidney disease [56]. Therefore, monitoring UA is crucial. One advantage of using saliva-based biosensors for UA measurement is that this procedure is less invasive than measuring the UA levels of the blood [50, 57–63]. Human saliva consists of various chemical substances, including amino acids, chloride, calcium, magnesium, and enzymes [64, 65], and is highly correlated with components found in the blood [60]. Moreover, various sensing platforms capable of processing saliva samples have been employed for UA detection. For example, Shi et al. developed an enzymatic biosensor for detecting UA in saliva samples using SPEs that are suitable for POCT applications [57]. To do so, they attached uricase to a working electrode fabricated using MWCNTs to enhance both sensitivity and selectivity. Figure 3e illustrates the fabrication process for this sensor. The optimized MWCNT and uricase sensor exhibited a broad linear range (5–1000 μmol/L) as well as a low detection limit (0.33 μmol/L), indicating that it can detect UA effectively. Further testing showed that this sensor successfully detected UA in both artificial saliva samples and real saliva samples from hyperuricemia patients, thereby demonstrating its potential for clinical applications involving POC health monitoring.

Another platform for sensing UA from saliva involves the use of a mouthguard sensor. Kim et al. developed a sensor in the form of a mouthguard capable of detecting salivary UA [60]. To do so, the authors modified a Prussian-blue graphite electrode by cross-linking with uricase, followed by electropolymerization with o-phenylenediamine, as shown in Fig. 3f. The resulting sensor not only successfully measured

UA in both artificial and undiluted real saliva samples but was also successful in measuring UA levels of hyperuricemia patients. Furthermore, the authors integrated miniaturized circuits for wireless data collection, thereby creating a wireless mouthguard product that is capable of continuously monitoring salivary UA. This application showcases the practicality of healthcare monitoring using the technologies discussed here.

Sensors for detecting other substances

The use of enzymatic biorecognition components makes it possible to sense many other substances in addition to glucose, lactate, and UA, including ethanol and cholesterol [66–70]. For example, Kim et al. used alcohol oxidase to detect ethanol levels using sweat samples, thereby creating a real-time, noninvasive system for monitoring alcohol consumption in everyday life [66]. Xu et al. developed a cholesterol sensor based on silver nanowires (AgNWs) modified with cholesterol oxidase (ChO_x) [67]. This sensor used CV for the clinical determination of cholesterol levels, and showed both high sensitivity and a low detection limit.

Recent research has begun to develop crucial biomarkers for the diagnosis of diseases in body fluids other than the blood, including sweat, tears, and saliva. The use of electrochemical sensors in diverse measurement environments suggests their usefulness as a tool for diagnosing disease by accurately and rapidly detecting biomarker concentrations without causing patient discomfort. In the future, new integrated sensor systems capable of simultaneously measuring multiple biomarkers and swiftly processing and interpreting large volumes of biological data can be coupled with advancements in flexible and safe materials, thus substantially enhancing biosensing technology.

Electrochemical immunosensors

Antibody-based electrochemical biosensors, also known as electrochemical immunosensors (EIs), are sensors capable of detecting specific immunoreaction interactions between an antibody and a target antigen. Furthermore, electrochemical immunosensors use changes in electron transfer rates caused by the immobilization of the target antigen on the antibody to detect biological reactions. Although EI-based methods have disadvantages, including batch-to-batch variation and high production costs, they also have important benefits, including high sensitivity and affordability, that make them well-suited for POCT applications [71, 72]. However, two important factors are necessary for their implementation: immobilization and amplification. In the following sections, we discuss the immobilization and amplification strategies of EI, as well as key applications of EI-based biosensing.

Immobilization of the receptor layer of electrochemical immunosensors

For EI biosensors, "immobilization" refers to the process of anchoring recognition elements—i.e., antibodies or antigens—on the electrode surface [73]. Immobilization is a critical step for EI since it enhances stability in the presence of oxidants, organic solvents, and digestive enzymes, thereby preserving biochemical functionality and ensuring that desired biosensor characteristics remain unchanged during downstream processing [74]. In general, immobilization requires maintaining the correct conformation and activity of specific proteins [75]. Methods for immobilization are typically categorized as belonging to one of two main groups: physical and chemical immobilization [76].

Physical immobilization

Physical adsorption is the simplest, quickest, and most widely used method of physical immobilization. It involves mechanical retention of a recognition element that is achieved via electrostatic interactions, hydrogen bonding, and/or van der Waals forces [73]. However, the weak bonding created by physical adsorption often results in antibodies having random orientations and weak attachments [74]. Another physical immobilization method involves developing new layers with different surface characteristics [77]. For example, in one case polymers were modified with various functional groups to facilitate stronger binding with antibodies [75].

Chemical immobilization

Chemical immobilization involves the formation of covalent bonds between the electrode surface and antibodies using bifunctional cross-linking reagents. This provides a stronger and more controllable attachment than is obtained from physical immobilization. Another advantage of chemical immobilization is that it permits control of the orientation of immobilized elements. Commonly used cross-linking reagents include glutaraldehyde [78], 3-aminopropyltriethoxysilane [79], and thiol derivatives [80]. However, when modifying electrodes using cross-linking reagents, the presence of a polymer layer can hinder electronic interactions between the redox marker and the electrode. Therefore, optimizing the process by using suitable modifications is crucial [74]. For example, Wei et al. [81] successfully achieved immobilization on ITO electrodes modified with (3-glycidoxypropyl)-trimethoxysilane (GPTMS). They did so by identifying optimal modification conditions that balanced the quantity of surface-bound antibodies and the electrochemical behavior of the redox label, and were able to achieve a detection limit of 10 nmol/L for benzo[*a*]pyrene.

Amplification of signals using electrochemical immunosensors

Many commercially available tools for immunochemical analysis are capable of highly precise measurements, with sensitivity limits approaching 1–100 pg/mL. However, these models are too expensive to be used for diagnosis and require lab-scale equipment, making them impractical for POCT applications [82]. Simple and compact sensors are required for diagnosing and monitoring diseases in various environments. However, these characteristics normally lead to aggravation in the sensing performance and detection limits. Therefore, further development of signal amplification techniques for specific target molecules is essential for successful disease diagnosis and for personalized monitoring. Signal amplification strategies can be categorized into three main approaches:

(i) Using enzymes to catalyze a selective amplification reaction;
(ii) Co-immobilizing antibodies and signal molecules using nanomaterial carriers;
(iii) Using the redox or enzyme activities of nanomaterials as an electroactive label.

Table 2 summarizes the advantages, disadvantages, and examples of different amplification methods.

Signal amplification using enzymes

Enzymes are commonly used for EI applications due to their rapid reaction rates and catalytic selectivity. They offer several advantages in terms of usability, sensitivity, and specificity compared to other methods, but also have important drawbacks, including a limited dynamic range, the requirement of a prolonged incubation time for signal acquisition, and can have low compatibility depending on the nature of the sample matrix [82]. Horseradish peroxidase (HRP), glucose oxidase, and alkaline phosphatase (ALP) are commonly used enzymes for EI applications. Although several methods have been tested, only increasing the number of enzymes involved in the reaction has been found to enhance sensitivity. In one study, Xiong et al. [83] used an HRP-functionalized envision antibody complex as an identifier to achieve highly sensitive detection of alpha-fetoprotein (AFP). Moreover, this study used an antibody–enzyme network structure to increase the number of enzymes participating in the reaction, which facilitated a linear range of 0.005–0.2 ng/mL and a low detection limit.

Enzymes used for amplification can also be integrated with additional signal amplification techniques, including chemical–chemical redox cycling. Enzyme products can regenerate reducing or oxidizing agents during redox cycling, thereby further enhancing sensitivity [84]. For example, Haque et al. [85] developed a novel method that combined enzymatic Ag deposition with chemical–chemical redox cycling using β-nicotinamide adenine dinucleotide. This method led to a significantly increased rate of Ag deposition compared to Ag deposition alone. In this study, the detection limit improved from 1 ng/mL with only enzymatic Ag deposition to 1 pg/mL when both methods were used, representing nearly a thousandfold enhancement in sensitivity. In addition, Lai et al. developed an electrochemical immunosensor that used chitosan–ferrocene (CS-Fc). Ferrocene can use redox reactions to transfer electrons to the electrode, thereby enhancing amplification. For a carcinoembryonic antigen, this approach increased the linear range fivefold at a detection limit of less than 0.5 pg/mL [86] (Fig. 4a).

Amplification methods like this can be readily used for practical POCT applications. For example, Min et al. [87] developed a POCT system for swift sepsis detection. They used antibody-conjugated magnetic beads to capture IL-3, and then enzyme-tagged detector antibodies catalyzed the oxidation of 3,3′,5,5′-tetramethylbenzidine with H_2O_2. This method, which incorporated smartphone data retrieval, increased the reaction speed by a factor of five and increased sensitivity by a factor of 10 relative to conventional enzyme-linked immunosorbent assay (ELISA)-based alternatives.

Use of nanomaterials as nanocarriers

Nanomaterials are optimal nanocarriers due to their high surface area-to-volume ratio, which allows for a high signal tracer loading capacity. Moreover, they are able to engage in multivalent affinity interactions—e.g., electrostatic and hydrophobic interactions—with proteins [82, 84]. This approach is simple and inexpensive, and does not require additional processes such as surface modifications. However, nanocarriers are difficult to form uniformly, a limitation that can adversely affect labeling. Therefore, an appropriate conjugation strategy must be implemented to improve performance [82]. Representative examples of nanocarriers include gold nanoparticles, silica nanoparticles, and graphene oxides.

Gold nanoparticles (Au NPs) are among the most frequently employed nanomaterials, since they exhibit excellent biocompatibility, high conductivity, high stability, and favorable catalytic activity. Due to these characteristics, Au NPs are used both as catalysts and carriers, as well as for other purposes. For example, Shen et al. designed an immunosensor for detecting procalcitonin (PCT) using N, N-bis(ferrocenoyl)-diaminoethane (Fc-Fc), β-cyclodextrins (β-CD), and poly(amidoamine) dendrimer-encapsulated Au nanoparticles (PAMAM-Au). In this immunosensor, PAMAM-Au served a dual role, acting as a nanocatalyst for catalyzing the oxidation of ascorbic acid and as a nanocarrier for immobilizing a significant quantity

Table 2 Comparison of electrochemical immunosensor amplification methods

Signal amplification method	Advantages	Disadvantages	Amplification label	Target	Detection limit	Reference
Use of enzymes	High usability High sensitivity and specificity	Limited dynamic range Long incubation time Low compatibility with the sample matrix	HRP ALP	AFP Creatine kinase-MB	2 pg/mL 1 pg/mL	[83] [84]
Use of nanomaterials as nanocarriers	No requirements for additional processes	Nanomaterials are difficult to form uniformly	Au NPs Silica NP GO	PCT PSA Phosphorylated p53	0.36 pg/mL 2.7 pg/mL 0.01 nmol/L	[88] [89] [90]
Use of nanomaterials as electroactive labels	High sensitivity Rapid reaction time	Reaction with the analyte in the absence of immunoreaction	CNTs Au NPs	AFP hCG	0.33 pg/mL 5 pg/mL	[93] [94]

HRP: horseradish peroxidase; ALP: alkaline phosphatase; NP: nanoparticle; GO: graphene oxide; CNTs: carbon nanotubes; AFP: alpha-fetoprotein; PCT: procalcitonin; PSA: prostate-specific antigen; hCG: human chorionic gonadotropin

of β-CD and secondary antibodies. This signal amplification strategy extended the detection range for PCT from 1.8 mg/mL to 500 ng/mL, and yielded a low detection limit of 0.36 pg/mL [88] (Fig. 4c).

Silica nanoparticles, and in particular mesoporous silica nanoparticles (MSN), have an extensive surface area, a consistent and adjustable pore size, and customizable surface properties. In one study, Lin et al. [89] used 3,9-Bis(3-aminopropyl)-2,4,8,10-tetraoxaspiro[5.5]undecane-functionalized mesoporous silica nanoparticles (MSN-Acetal) to detect prostate-specific antigen (PSA). In addition, they used thionine (Th) as an electron mediator, capping it within the pores and designing it to be released under acidic conditions. This method yielded a very low detection concentration of 2.7 pg/mL and an extensive operational range between 3.0 pg/mL and 20 ng/mL.

Graphene oxide is another nanocarrier, and is commonly used due to its high loading capacity. In one study, Du et al. [90] developed an immunosensor using a multienzyme amplification strategy combined with graphene oxide to detect p53 that was phosphorylated at Ser392. They enhanced sensitivity by attaching bioconjugates containing HRP and the p53(392) signal antibody to functionalized graphene oxide. This approach achieved a linear range of 0.02–0.2 nmol/L and a low detection limit of 0.01 nmol/L, which is 10 times better than the limit obtained using traditional sandwich electrochemical measurement techniques.

Furthermore, there are cases in which amplification methods using nanomaterials as nanocarriers have been applied to POCT. For example, Arévalo et al. [91] presented a small sandwich-type immunoassay using camelid antibodies (cAbs) for the simultaneous determination of two important immunity-related cytokines, BAFF (i.e., a B cell activation factor) and APRIL (i.e., a proliferation-induced signal). In this study, for signal amplification, the detection antibodies (dAbs) were labeled with binary MoS_2/MWCNT nanostructures, and HRP was used for reporting. As a result, the detection limits obtained were 0.08 ng/mL for BAFF and 0.06 ng/mL for APRIL, and these values were consistent with measurements obtained using traditional ELISA techniques.

Use of nanomaterials as electroactive labels

Nanomaterials, especially metal nanoparticles, can be used as electroactive labels in many applications, since they increase the number of electroactive species by accumulating signal molecules on the electrode surface [92]. This technique uses direct redox reactions, and leads to high sensitivity and short reaction time. However, since nanomaterials can react even in the absence of immunoreactions, careful analyte selection is crucial. Carbon nanotubes (CNTs) and Au NPs are the most commonly used nanomaterials used as electroactive labels.

CNTs are employed as electroactive labels due to their outstanding electronic characteristics and high surface area for protein covalent bonding. For example, Jiao et al. [93] developed an immunosensor to enhance the sensitivity of AFP by using poly-dopamine functionalized N-doped multiwalled carbon nanotubes (PDA-N-MWCNTs) along with a

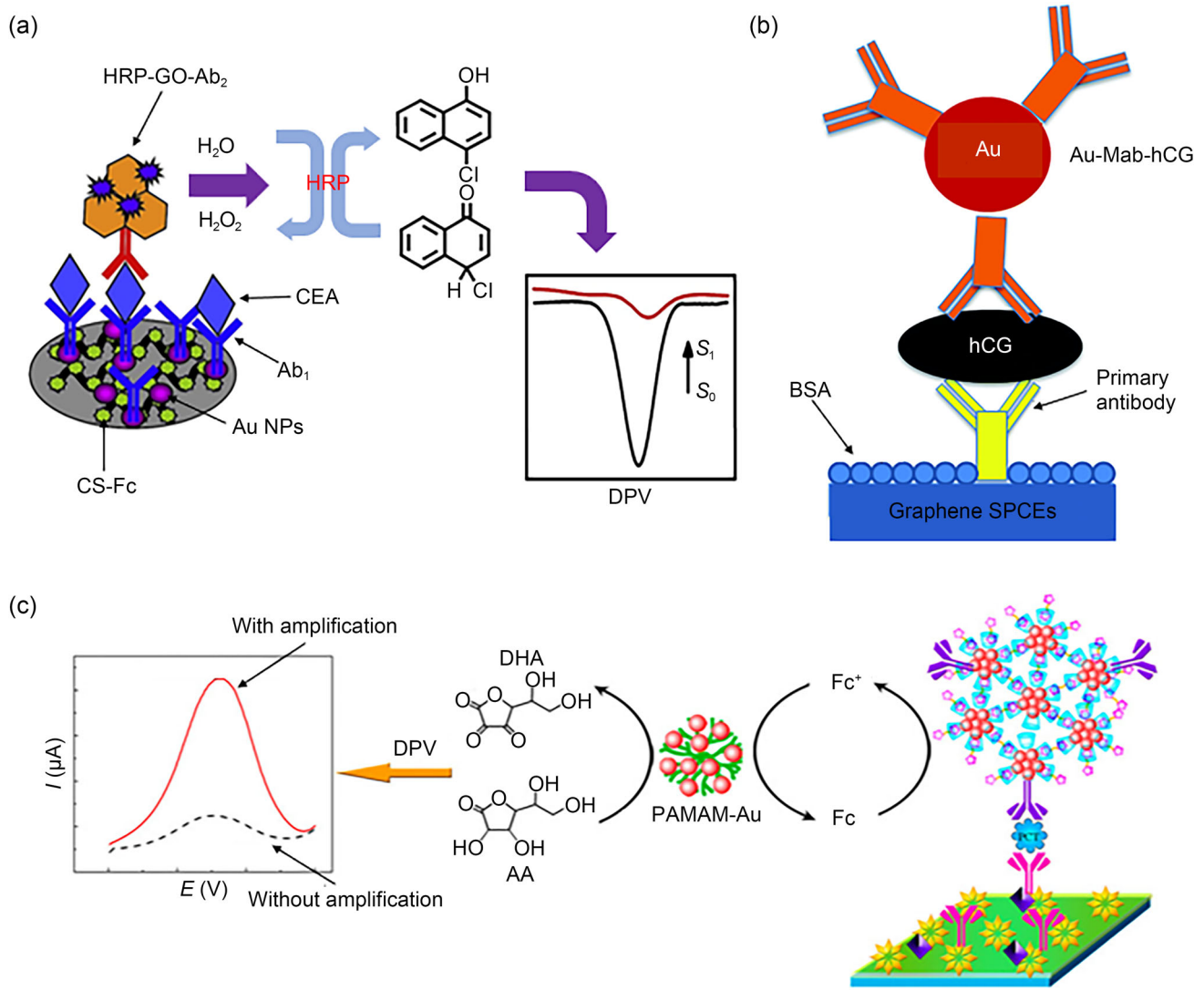

Fig. 4 **a** Schematic representation of an electrochemical detection strategy using enzyme-functionalized graphene oxide (GO) (reproduced from Ref. [86], Copyright 2015, with permission from Elsevier). **b** A redox reaction was performed in an HCl solution to initiate Au NP oxidation (reproduced from Ref. [94], Copyright 2014, with permission from The Royal Society of Chemistry). **c** The dual functions of poly(amidoamine) dendrimer-encapsulated Au nanoparticles (PAMAM-Au) as nanocatalysts for catalyzing the oxidation of ascorbic acid and as nanocarriers for anchoring β-cyclodextrins (β-CD) and secondary antibodies (reproduced from Ref. [88], Copyright 2015, with permission from the American Chemical Society)

graphene-loaded mesoporous Au@Pt nanocomposite. PDA-N-MWCNTs, which have a large surface area, provide a broad electroactive area for antibodies, exhibit high conductivity, and have high electrocatalytic activity, achieving a linear range of 10 pg/mL–20 ng/mL and a low detection limit of 0.33 pg/mL.

Au NPs are also widely used metal nanoparticles due to their straightforward manufacturing process and high stability relative to other metal nanomaterials. They also permit efficient bioconjugation. For example, Lim et al. introduced an immunosensor for measuring human chorionic gonadotropin (hCG) using Au NPs as electrochemical labels and graphene as the electrode material. The authors first immobilized the primary antibody on the graphene working electrode via physical adsorption, added the hCG antigen, and then created a sandwich structure using a secondary antibody labeled with Au NPs. This sandwich-type biosensor attained a low detection limit of 5 pg/mL [94] (Fig. 4b).

Amplification using nanomaterials as electroactive labels is also frequently used for POCT applications. For example, Liu et al. [95] introduced a disposable electrochemical immunosensor diagnostic device that uses Cd, Zn, and quantum dots as labels for amplification. This device was able to successfully detect IgG at a concentration of 30 pg/mL within 7 min and was able to successfully detect PSA in human serum samples. Moreover, all of these results showed high

consistency with existing ELISA techniques. This portable device therefore shows great promise for quantitative testing of disease-related protein biomarkers at the POC.

Applications of electrochemical immunosensors to POC devices

Advances in microelectronic technology have enabled the fabrication of microelectrodes, which facilitates the measurement of very small sample volumes. Furthermore, it is known that volume does not significantly affect the sensitivity of electrochemical methods [96]. Electrochemical immunosensors are highly optimized for small, portable POC devices due to their relatively high sensitivity. These features have led to the widespread application of electrochemical antibody-based biosensors for various POCT applications.

One prominent field of application is disease diagnosis. Compared to traditional, time-consuming, and expensive laboratory-based immunoassays, portable immunosensors offer a cost-effective and straightforward way to diagnose disease. Dutta and Lillehoj introduced immunosensors for detecting the *Plasmodium falciparum* histidine-rich protein 2 (*Pf* HRP2), which is used in rapid diagnostic tests for malaria diagnosis. These immunosensors were manufactured using a simple process in which all reagents were dry-stacked in a membrane assembly. The measurement process requires only two liquid dispensing steps, thereby making it possible to obtain results within 5 min with a linear range of 100 ng/mL to 100 μg/mL [97] (Fig. 5a).

Immunosensors have also been employed to monitor the activities of cells or tissues through hormones such as cortisol and lactate. For example, Tuteja et al. presented a POC platform that measures cortisol and lactate levels in body fluids such as saliva and sweat. In that study, they used antibodies bioconjugated to electroreduced graphene oxide (e-RGO) to enable the simultaneous measurement of cortisol and lactate levels [98] (Fig. 5b). Their results showed a strong correlation with commercial ELISA kits and permitted accurate quantification within 1 min. Khan et al. [99] developed an electrochemical-digital sensor chip capable of on-site recording of cortisol levels. They then used a high-accuracy impedance converter system and EIS to accurately measure cortisol levels in saliva. This biosensor chip achieved a low detection limit of 0.9 pg/mL.

Immunosensors have also been used to detect cardiac markers to assess heart damage, stress, or inflammation. For example, Kamakoti et al. introduced a biosensor that uses molybdenum as a transition metal electrode and polyamide as a substrate to sense cardiac Troponin-I (cTnI). The flexible polyamide substrate and small device size reported in this study suggest the possibility for use in textile-based POC diagnostic platforms. This biosensor achieved detection limits for cTnI of 10 pg/mL in PBS and 1 ng/mL in HS medium [100] (Fig. 5c).

Other electrochemical biosensors

In addition to the enzyme- and antibody-based biosensors reported above, other methods, including aptamers and molecularly imprinted polymers (MIPs), are being screened for the ability to identify biomolecules, and this can also facilitate the development of novel POC devices. Aptamer-based biosensors, known as aptasensors, have recently emerged as a method for detecting various biomolecules. Aptamers are short, single-stranded DNA or RNA molecules that are capable of selectively binding to a diverse range of targets, including proteins and nucleic acids, using a procedure known as Systematic Evolution of Ligands by EXponential enrichment (SELEX) [101]. In addition, electrochemical aptamer-based biosensors may be suitable for deployment in POC devices due to their low cost, high sensitivity, and high selectivity. They can also be used as biomarkers for proteins [102, 103] and viruses [104]. However, these sensors also have important limitations, including susceptibility to ionic interference from biospecimens and difficulty in aptamer generation. MIP-based biosensors were first proposed by Wulff in 1995. To construct these sensors, target molecules are present during synthesis, and the correct arrangement is induced using monomers and template molecules. Free receptor sites are then created via a washing step, and this enables target recognition. This method can be employed using a broad spectrum of materials, including carbon-based materials such as carbon nanotubes [105] and graphene [106] as well as metal oxides [107]. However, MIP-based biosensors have limitations, including poor sensitivity, a poor detection limit, mechanical compliance issues, and sensor instability. Each of these limitations should be addressed by future research.

Conclusions

In this review, we examined various electrochemical biosensors for POCT applications using enzymes, antibodies, aptamers, MIPs, and others. Table 3 summarizes the advantages, disadvantages, and potential applications of the electrochemical biosensors discussed. Successful adoption of POC devices requires several conditions, including affordability, effective miniaturization, high sensitivity, and short measurement time; electrochemical biosensors are

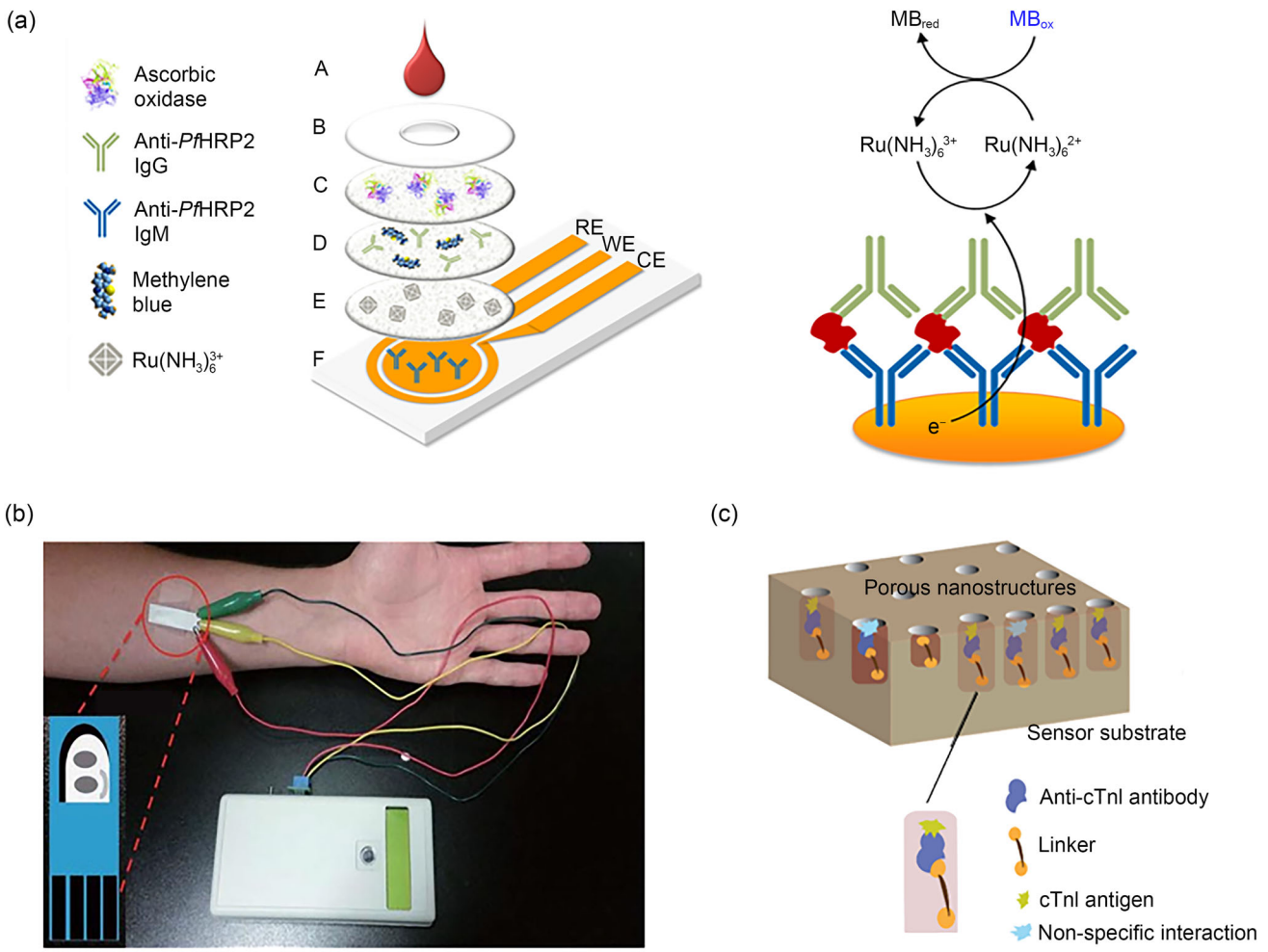

Fig. 5 **a** Schematic representation of an immunoassay for the rapid electrochemical detection of *Plasmodium falciparum* histidine-rich protein 2 (*Pf* HRP2) in blood (reproduced from Ref. [97], Copyright 2018, with permission from the authors, licensed under CC BY 4.0). **b** Schematic representation of a portable, handheld potentiostat implemented in a point-of-care (POC) platform capable of measuring cortisol and lactate in biofluids (reproduced from Ref. [98], Copyright 2018, with permission from the authors, licensed under CC BY). **c** Schematic representation of an immunoassay using an Mo biosensor (reproduced from Ref. [100], Copyright 2016, with permission from the authors, licensed under CC BY 4.0)

therefore an optimal choice based on these criteria. Numerous pioneering studies have been conducted on biosensors using a variety of biorecognition elements. Moreover, recent advancements in fabrication technologies have enabled further device miniaturization, which has made it easier to produce devices suitable for POCT applications. Electrochemical enzyme-based biosensors are useful for detecting and quantifying analytes at low concentrations due to their ability to exploit specific interactions with target molecules or chemical substances, and their ability to provide highly sensitive measurements. Furthermore, enzymes exhibit high selectivity, thereby allowing precise detection of the desired analyte while minimizing interference from other chemical substances. Electrochemical antibody-based biosensors use immunoreaction interactions between antibodies and their target antigens to generate a signal. This interaction offers excellent specificity, and thereby allows only the monitoring of desired reactions. However, there are also ongoing research programs that use aptamers, MIPs, and other biorecognition elements. While many of these specific techniques have yet to be commercialized, the demand for POC devices is anticipated to continue to increase. To date, a high proportion of research has focused on measurement techniques. However, for effective commercialization, additional research should be conducted to integrate biosensors with other devices, make them even smaller, and make the measurement process more convenient. Overall, the use of novel micro- and nanotechnologies has considerable potential for further development. If successful, the deployment of the biosensors discussed here may help patients with various diseases to be able to obtain precise analysis results in just a few minutes within their own homes.

Table 3 Comparison of biological recognition units used in electrochemical sensor applications

Biological recognition unit	Advantages	Disadvantages	Target	Detection limit	POC application	Reference
Enzyme	High sensitivity and selectivity Low cost Short response time Low detection limit	Enzymes can be unstable Enzyme immobilization is difficult	Glucose	5–500 μmol/L	Measuring glucose levels	[31–37]
			Lactate acid	1 mmol/L	Determining the concentration of lactate acid	[45–48]
			Uric acid	0.1–100 μmol/L	Measuring uric acid levels	[50, 57–63]
Antibody	High sensitivity Affordable	Batch-to-batch variation High production cost	*Pf*HRP2	100 ng/mL	Diagnostic tests for malaria	[97]
			Cortisol	0.87 pg/mL	Measuring cortisol levels in saliva	[99]
			cTnI	10 pg/mL	Detecting cardiac markers using a textile-based platform	[100]
Aptamer	High sensitivity and selectivity Low cost	Difficulty in generating aptamers Susceptibility to ionic interference	Thrombin	267 fmol/L	Real-time detection of thrombin	[103]
MIP	Employed with a broad spectrum of materials	Low sensitivity and detection limit Instability	Urea	500 nmol/L	Urea quantitation in real samples	[105]

POCT: point-of-care testing; MIP: molecularly imprinted polymer; PfHRP2: *Plasmodium falciparum* histidine-rich protein 2; cTnI: cardiac Troponin-I

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Author contributions JK and JJ were involved in organizing the paper, formal analysis, conceptualization, writing the original draft, and doing the commentary editing. SHK was involved in funding acquisition, project administration, and reviewing the written draft of the paper.

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Declarations

Conflict of interest This paper is to be included in a special issue for which SHK is a guest editor. SHK is also an associate editor for *Bio-Design and Manufacturing*. He was not involved in the editorial review or the decision to publish this article. The authors declare that they have no conflict of interest.

Ethical approval This study does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Campuzano S, Pedrero M, Yáñez-Sedeño P et al (2021) New challenges in point of care electrochemical detection of clinical biomarkers. Sens Actuat B Chem 345:130349. https://doi.org/10.1016/j.snb.2021.130349
2. Avraham R (2006) Moving biosensors to point-of-care cancer diagnostics. Biosens Bioelectron 21(10):1847–1850. https://doi.org/10.1016/j.bios.2006.02.001
3. Wang J (2006) Electrochemical biosensors: towards point-of-care cancer diagnostics. Biosens Bioelectron 21(10):1887–1892. https://doi.org/10.1016/j.bios.2005.10.027
4. da Silva ETSG, Souto DEP, Barragan JTC et al (2017) Electrochemical biosensors in point-of-care devices: recent advances and future trends. ChemElectroChem 4(4):778–794. https://doi.org/10.1002/celc.201600758
5. Dai YF, Liu CC (2019) Recent advances on electrochemical biosensing strategies toward universal point-of-care systems. Angew Chem 131(36):12483–12496. https://doi.org/10.1002/ange.201901879

6. Yu Y, Nyein HYY, Gao W et al (2020) Flexible electrochemical bioelectronics: the rise of in situ bioanalysis. Adv Mater 32(15):e1902083. https://doi.org/10.1002/adma.201902083
7. Ronkainen NJ, Halsall HB, Heineman WR (2010) Electrochemical biosensors. Chem Soc Rev 39(5):1747–1763. https://doi.org/10.1039/b714449k
8. Rocchitta G, Spanu A, Babudieri S et al (2016) Enzyme biosensors for biomedical applications: strategies for safeguarding analytical performances in biological fluids. Sensors 16(6):780. https://doi.org/10.3390/s16060780
9. Sumitha MS, Xavier TS (2023) Recent advances in electrochemical biosensors—a brief review. Hybrid Adv 2:100023. https://doi.org/10.1016/j.hybadv.2023.100023
10. Nguyen HH, Kim M (2017) An overview of techniques in enzyme immobilization. Appl Sci Converg Technol 26(6):157–163. https://doi.org/10.5757/ASCT.2017.26.6.157
11. Liu XW, Hu QY, Wu Q et al (2009) Aligned ZnO nanorods: a useful film to fabricate amperometric glucose biosensor. Colloids Surf B 74(1):154–158. https://doi.org/10.1016/j.colsurfb.2009.07.011
12. Kowalewska B, Jakubow K (2017) The impact of immobilization process on the electrochemical performance, bioactivity and conformation of glucose oxidase enzyme. Sens Actuat B Chem 238:852–861. https://doi.org/10.1016/j.snb.2016.07.138
13. Barredo JL (2005) Microbial Enzymes and Biotransformations. Humana Totowa, NJ, USA. https://doi.org/10.1385/1592598463
14. Sakalauskiene L, Popov A, Kausaite-Minkstimiene A et al (2022) The impact of glucose oxidase immobilization on dendritic gold nanostructures on the performance of glucose biosensors. Biosensors 12(5):320. https://doi.org/10.3390/bios12050320
15. Wang Y, Zhai FG, Hasebe Y et al (2018) A highly sensitive electrochemical biosensor for phenol derivatives using a graphene oxide-modified tyrosinase electrode. Bioelectrochemistry 122:174–182. https://doi.org/10.1016/j.bioelechem.2018.04.003
16. Bi R, Ma XY, Miao KP et al (2023) Enzymatic biosensor based on dendritic gold nanostructure and enzyme precipitation coating for glucose sensing and detection. Enzyme Microb Technol 162:110132. https://doi.org/10.1016/j.enzmictec.2022.110132
17. Sharma D, Lee J, Seo J et al (2017) Development of a sensitive electrochemical enzymatic reaction-based cholesterol biosensor using nano-sized carbon interdigitated electrodes decorated with gold nanoparticles. Sensors 17(9):2128. https://doi.org/10.3390/s17092128
18. Yan QH, Zhi N, Yang L et al (2020) A highly sensitive uric acid electrochemical biosensor based on a nano-cube cuprous oxide/ferrocene/uricase modified glassy carbon electrode. Sci Rep 10(1):10607. https://doi.org/10.1038/s41598-020-67394-8
19. Artigues M, Gilabert-Porres J, Texidó R et al (2021) Analytical parameters of a novel glucose biosensor based on grafted PFM as a covalent immobilization technique. Sensors 21(12):4185. https://doi.org/10.3390/s21124185
20. Baluta S, Lesiak A, Cabaj J (2020) Simple and cost-effective electrochemical method for norepinephrine determination based on carbon dots and tyrosinase. Sensors 20(16):4567. https://doi.org/10.3390/s20164567
21. Broun GB (1976) Chemically aggregated enzymes. Method Enzymol 44(5):263–280. https://doi.org/10.1016/S0076-6879(76)44022-3
22. Yan L, Miao KP, Ma PC et al (2021) A feasible electrochemical biosensor for determination of glucose based on Prussian blue – enzyme aggregates cascade catalytic system. Bioelectrochemistry 141:107838. https://doi.org/10.1016/j.bioelechem.2021.107838
23. Hegedus I, Nagy E (2015) Stabilization of activity of cellulase and hemicellulase enzymes by covering with polyacrylamide layer. Chem Eng Process 95:143–150. https://doi.org/10.1016/j.cep.2015.06.005
24. Chapman R, Stenzel MH (2019) All wrapped up: stabilization of enzymes within single enzyme nanoparticles. J Am Chem Soc 141(7):2754–2769. https://doi.org/10.1021/jacs.8b10338
25. Hegedüs I, Hancsók J, Nagy E (2012) Stabilization of the cellulase enzyme complex as enzyme nanoparticle. Appl Biochem Biotechnol 168(6):1372–1383. https://doi.org/10.1007/s12010-012-9863-9
26. Kim J, Grate JW (2003) Single-enzyme nanoparticles armored by a nanometer-scale organic/inorganic network. Nano Lett 3(9):1219–1222. https://doi.org/10.1021/nl034404b
27. Zhong LT, Zhai JQ, Ma Y et al (2022) Molecularly imprinted polymers with enzymatic properties reduce cytokine release syndrome. ACS Nano 16(3):3797–3807. https://doi.org/10.1021/acsnano.1c08297
28. Ciui B, Tertis M, Florea A et al (2017) Electrochemical sensor for dopamine based on electropolymerized molecularly imprinted poly-aminothiophenol and gold nanoparticles. Proc Technol 27:118–119. https://doi.org/10.1016/j.protcy.2017.04.052
29. Rassas I, Braiek M, Bonhomme A et al (2019) Voltammetric glucose biosensor based on glucose oxidase encapsulation in a chitosan-kappa-carrageenan polyelectrolyte complex. Mater Sci Eng C 95:152–159. https://doi.org/10.1016/j.msec.2018.10.078
30. Oliver NS, Toumazou C, Cass AEG et al (2009) Glucose sensors: a review of current and emerging technology. Diabet Med 26(3):197–210. https://doi.org/10.1111/j.1464-5491.2008.02642.x
31. Lee H, Choi TK, Lee YB et al (2016) A graphene-based electrochemical device with thermoresponsive microneedles for diabetes monitoring and therapy. Nat Nanotech 11(6):566–572. https://doi.org/10.1038/nnano.2016.38
32. Chen YH, Lu SY, Zhang SS et al (2017) Skin-like biosensor system via electrochemical channels for non-invasive blood glucose monitoring. Sci Adv 3(12):e1701629. https://doi.org/10.1126/sciadv.1701629
33. Lee H, Song C, Hong YS et al (2017) Wearable/disposable sweat-based glucose monitoring device with multistage transdermal drug delivery module. Sci Adv 3(3):e1601314. https://doi.org/10.1126/sciadv.1601314
34. Keum DH, Kim SK, Koo J et al (2020) Wireless smart contact lens for diabetic diagnosis and therapy. Sci Adv 6(17):eaba3252. https://doi.org/10.1126/sciadv.aba3252
35. Gao W, Emaminejad S, Nyein HYY et al (2016) Fully integrated wearable sensor arrays for multiplexed in situ perspiration analysis. Nature 529(7587):509–514. https://doi.org/10.1038/nature16521
36. Soni A, Jha SK (2015) A paper strip based non-invasive glucose biosensor for salivary analysis. Biosens Bioelectron 67:763–768. https://doi.org/10.1016/j.bios.2014.09.042
37. Iguchi S, Kudo H, Saito T (2007) A flexible and wearable biosensor for tear glucose measurement. Biomed Microdevices 9(4):603–609. https://doi.org/10.1007/s10544-007-9073-3
38. Dartt DA, Hodges RR, Zoukhri D (2005) Tears and their secretion. Adv Environ Biol 10:21–82. https://doi.org/10.1016/S1569-2590(05)10002-0
39. Wan JJ, Qin Z, Wang PY et al (2017) Muscle fatigue: general understanding and treatment. Exp Mol Med 49(10):e384. https://doi.org/10.1038/emm.2017.194
40. Finsterer J (2012) Biomarkers of peripheral muscle fatigue during exercise. BMC Musculoskelet Disord 13(1):218. https://doi.org/10.1186/1471-2474-13-218
41. Alam F, RoyChoudhury S, Jalal AH et al (2018) Lactate biosensing: the emerging point-of-care and personal health monitoring. Biosens Bioelectron 117:818–829. https://doi.org/10.1016/j.bios.2018.06.054
42. Kim S, Yang WS, Kim HJ et al (2019) Highly sensitive non-enzymatic lactate biosensor driven by porous nanostructured

nickel oxide. Ceram Int 45(17):23370–23376. https://doi.org/10.1016/j.ceramint.2019.08.037
43. Shakhih MFM, Rosslan AS, Noor AM et al (2021) Review-enzymatic and non-enzymatic electrochemical sensor for lactate detection in human biofluids. J Electrochem Soc 168(6):067502. https://doi.org/10.1149/1945-7111/ac0360
44. Karpova EV, Laptev AI, Andreev EA et al (2020) Relationship between sweat and blood lactate levels during exhaustive physical exercise. ChemElectroChem 7(1):191–194. https://doi.org/10.1002/celc.201901703
45. Jia WZ, Bandodkar AJ, Valdés-Ramírez G (2013) Electrochemical tattoo biosensors for real-time noninvasive lactate monitoring in human perspiration. Anal Chem 85(14):6553–6560. https://doi.org/10.1021/ac401573r
46. Saha T, Songkakul T, Knisely CT et al (2022) Wireless wearable electrochemical sensing platform with zero-power osmotic sweat extraction for continuous lactate monitoring. ACS Sens 7(7):2037–2048. https://doi.org/10.1021/acssensors.2c00830
47. Hojaiji H, Zhao YC, Gong MC et al (2020) An autonomous wearable system for diurnal sweat biomarker data acquisition. Lab Chip 20(24):4582–4591. https://doi.org/10.1039/D0LC00820F
48. Xuan X, Perez-Rafols C, Chen C et al (2021) Lactate biosensing for reliable on-body sweat analysis. ACS Sens 6(7):2763–2771. https://doi.org/10.1021/acssensors.1c01009
49. Sun T, Hui JN, Zhou L et al (2022) A low-cost and simple-fabricated epidermal sweat patch based on "cut-and-paste" manufacture. Sens Actuat B Chem 368:132184. https://doi.org/10.1016/j.snb.2022.132184
50. Piedras J, Dominguez RB, Gutiérrez JM (2021) Determination of uric acid in artificial saliva with compact AMP3291 reader and Au nanoparticles modified electrode. Chemosens 9(4):73. https://doi.org/10.3390/chemosensors9040073
51. Moran ME (2003) Uric acid stone disease. Front Biosci 8(6):s1339–s1355. https://doi.org/10.2741/1178
52. Falasca GF (2006) Metabolic diseases: gout. Clin Dermatol 24(6):498–508. https://doi.org/10.1016/j.clindermatol.2006.07.015
53. Nyhan WL (1997) The recognition of Lesch-Nyhan syndrome as an inborn error of purine metabolism. J Inherit Metab Dis 20(2):171–178. https://doi.org/10.1023/A:1005348504512
54. Puig JG, Martinez MA (2008) Hyperuricemia, gout and the metabolic syndrome. Curr Opin Rheumatol 20(2):187–191. https://doi.org/10.1097/BOR.0b013e3282f4b1ed
55. Chang CC, Wu CH, Liu LK et al (2018) Association between serum uric acid and cardiovascular risk in nonhypertensive and nondiabetic individuals: the Taiwan I-Lan longitudinal aging study. Sci Rep 8(1):5234. https://doi.org/10.1038/s41598-018-22997-0
56. Miyaoka T, Mochizuki T, Takei T et al (2013) Serum uric acid levels and long-term outcomes in chronic kidney disease. Heart Vessels 29(4):504–512. https://doi.org/10.1007/s00380-013-0396-0
57. Shi WS, Li J, Wu J et al (2020) An electrochemical biosensor based on multi-wall carbon nanotube-modified screen-printed electrode immobilized by uricase for the detection of salivary uric acid. Anal Bioanal Chem 412(26):7275–7283. https://doi.org/10.1007/s00216-020-02860-w
58. Liu ML, Chen Q, Lai CL et al (2013) A double signal amplification platform for ultrasensitive and simultaneous detection of ascorbic acid, dopamine, uric acid and acetaminophen based on a nanocomposite of ferrocene thiolate stabilized Fe_3O_4@Au nanoparticles with graphene sheet. Biosens Bioelectron 48:75–81. https://doi.org/10.1016/j.bios.2013.03.070
59. Yang LQ, Huang N, Lu QJ et al (2016) A quadruplet electrochemical platform for ultrasensitive and simultaneous detection of ascorbic acid, dopamine, uric acid and acetaminophen based on a ferrocene derivative functional Au NPs/carbon dots nanocomposite and graphene. Anal Chim Acta 903:69–80. https://doi.org/10.1016/j.aca.2015.11.021
60. Kim J, Imani S, de Araujo WR et al (2015) Wearable salivary uric acid mouthguard biosensor with integrated wireless electronics. Biosens Bioelectron 74:1061–1068. https://doi.org/10.1016/j.bios.2015.07.039
61. Ngamchuea K, Batchelor-McAuley C, Compton RG (2018) Understanding electroanalytical measurements in authentic human saliva leading to the detection of salivary uric acid. Sens Actuat B Chem 262:404–410. https://doi.org/10.1016/j.snb.2018.02.014
62. Turkkan G, Bas SZ, Atacan K et al (2022) An electrochemical sensor based on a Co_3O_4–ERGO nanocomposite modified screen-printed electrode for detection of uric acid in artificial saliva. Anal Methods 14(1):67–75. https://doi.org/10.1039/d1ay01744f
63. Huang X, Shi WS, Li J et al (2020) Determination of salivary uric acid by using poly(3,4-ethylenedioxythipohene) and graphene oxide in a disposable paper-based analytical device. Anal Chim Acta 1103:75–83. https://doi.org/10.1016/j.aca.2019.12.057
64. Pink R, Simek J, Vondrakova J et al (2009) Saliva as a diagnostic medium. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 153(2):103–110. https://doi.org/10.5507/bp.2009.017
65. Ilea A, Andrei V, Feurdean CN et al (2019) Saliva, a magic biofluid available for multilevel assessment and a mirror of general health—a systematic review. Biosensors 9(1):27. https://doi.org/10.3390/bios9010027
66. Kim J, Jeerapan I, Imani S et al (2016) Non-invasive alcohol monitoring using a wearable tattoo-based iontophoretic-biosensing system. ACS Sens 1(8):1011–1019. https://doi.org/10.1021/acssensors.6b00356
67. Xu L, Hou YT, Zhang MD et al (2015) Electrochemical sensor based on a silver nanowires modified electrode for the determination of cholesterol. Anal Methods 7(13):5649–5653. https://doi.org/10.1039/C5AY01164G
68. Ahmadraji T, Killard AJ (2016) Measurement of total cholesterol using an enzyme sensor based on a printed hydrogen peroxide electrocatalyst. Anal Methods 8(13):2743–2749. https://doi.org/10.1039/C6AY00468G
69. Lin XY, Ni YN, Kokot S (2016) Electrochemical cholesterol sensor based on cholesterol oxidase and MoS_2-AuNPs modified glassy carbon electrode. Sens Actuat B Chem 233:100–106. https://doi.org/10.1016/j.snb.2016.04.019
70. Sun YF, Nguyen TN, Anderson A et al (2020) In vivo glutamate sensing inside the mouse brain with perovskite nickelate−Nafion heterostructures. ACS Appl Mater Interfaces 12(22):24564–24574. https://doi.org/10.1021/acsami.0c02826
71. Tu JB, Torrente-Rodríguez RM, Wang MQ et al (2020) The era of digital health: a review of portable and wearable affinity biosensors. Adv Funct Mater 30(29):2070197. https://doi.org/10.1002/adfm.201906713
72. Aydin M, Aydin EB, Sezgintürk MK (2021) Advances in immunosensor technology. Adv Clin Chem 102:1–62. https://doi.org/10.1016/bs.acc.2020.08.001
73. Mollarasouli F, Kurbanoglu S, Ozkan SA (2019) The role of electrochemical immunosensors in clinical analysis. Biosensors 9(3):86. https://doi.org/10.3390/bios9030086
74. Evtugyn G (2014) Biosensors: Essentials. Springer, Berlin, p. 261. https://doi.org/10.1007/978-3-642-40241-8
75. Wan Y, Su Y, Zhu XH et al (2013) Development of electrochemical immunosensors towards point of care diagnostics. Biosens Bioelectron 47:1–11. https://doi.org/10.1016/j.bios.2013.02.045
76. Svalova TS, Malysheva NN, Kozitsina AN (2017) Structure of the receptor layer in electrochemical immunosensors. Modern trends and prospects of development. Russian Chem Bull 66(10):1797–1811. https://doi.org/10.1007/s11172-017-1951-0

77. Cong QM, Bian HM, Yu ZX et al (2015) A reagentless electrochemical immunosensor based on probe immobilization and the layer-by-layer assembly technique for sensitive detection of tumor markers. Anal Methods 7(22):9655–9662. https://doi.org/10.1039/c5ay01871d
78. Jiang DC, Tang J, Liu BH et al (2003) Covalently coupling the antibody on an amine-self-assembled gold surface to probe hyaluronan-binding protein with capacitance measurement. Biosens Bioelectron 18(9):1183–1191. https://doi.org/10.1016/S0956-5663(02)00253-1
79. Vashist SK, Lam E, Hrapovic S et al (2014) Immobilization of antibodies and enzymes on 3-aminopropyltriethoxysilane-functionalized bioanalytical platforms for biosensors and diagnostics. Chem Rev 114(21):11083–11130. https://doi.org/10.1021/cr5000943
80. Ahmad A, Moore E (2012) Electrochemical immunosensor modified with self-assembled monolayer of 11-mercaptoundecanoic acid on gold electrodes for detection of benzo[*a*]pyrene in water. Analyst 137(24):5839–5844. https://doi.org/10.1039/C2AN35236B
81. Wei MY, Wen SD, Yang XQ et al (2009) Development of redox-labeled electrochemical immunoassay for polycyclic aromatic hydrocarbons with controlled surface modification and catalytic voltammetric detection. Biosens Bioelectron 24(9):2909–2914. https://doi.org/10.1016/j.bios.2009.02.031
82. Cho IH, Lee J, Kim J et al (2018) Current technologies of electrochemical immunosensors: perspective on signal amplification. Sensors 18(1):207. https://doi.org/10.3390/s18010207
83. Xiong P, Gan N, Cao YT et al (2012) An ultrasensitive electrochemical immunosensor for alpha-fetoprotein using an envision complex-antibody copolymer as a sensitive label. Materials 5(12):2757–2772. https://doi.org/10.3390/ma5122757
84. Wen W, Yan X, Zhu CZ et al (2017) Recent advances in electrochemical immunosensors. Anal Chem 89(1):138–156. https://doi.org/10.1021/acs.analchem.6b04281
85. Haque AM, Kim J, Dutta G et al (2015) Redox cycling-amplified enzymatic Ag deposition and its application in the highly sensitive detection of creatine kinase-MB. Chem Commun 51(77):14493–14496. https://doi.org/10.1039/C5CC06117B
86. Lai GS, Cheng H, Xin DH et al (2016) Amplified inhibition of the electrochemical signal of ferrocene by enzyme-functionalized graphene oxide nanoprobe for ultrasensitive immunoassay. Anal Chim Acta 902:189–195. https://doi.org/10.1016/j.aca.2015.11.014
87. Min J, Nothing M, Coble B et al (2018) Integrated biosensor for rapid and point-of-care sepsis diagnosis. ACS Nano 12(4):3378–3384. https://doi.org/10.1021/acsnano.7b08965
88. Shen WJ, Zhuo Y, Chai YQ et al (2015) Enzyme-free electrochemical immunosensor based on host–guest nanonets catalyzing amplification for procalcitonin detection. ACS Appl Mater Interfaces 7(7):4127–4134. https://doi.org/10.1021/am508137t
89. Lin YX, Zhou Q, Lin YP et al (2015) Enzymatic hydrolysate-induced displacement reaction with multifunctional silica beads doped with horseradish peroxidase–thionine conjugate for ultrasensitive electrochemical immunoassay. Anal Chem 87(16):8531–8540. https://doi.org/10.1021/acs.analchem.5b02253
90. Du D, Wang LM, Shao YY et al (2011) Functionalized graphene oxide as a nanocarrier in a multienzyme labeling amplification strategy for ultrasensitive electrochemical immunoassay of phosphorylated p53 (S392). Anal Chem 83(3):746–752. https://doi.org/10.1021/ac101715s
91. Arévalo B, Blázquez-García M, Valverde A et al (2022) Binary MoS_2 nanostructures as nanocarriers for amplification in multiplexed electrochemical immunosensing: simultaneous determination of B cell activation factor and proliferation-induced signal immunity-related cytokines. Mikrochim Acta 189(4):143. https://doi.org/10.1007/s00604-022-05250-4
92. Lim SA, Ahmed MU (2016) Electrochemical immunosensors and their recent nanomaterial-based signal amplification strategies: a review. RSC Adv 6(30):24995–25014. https://doi.org/10.1039/C6RA00333H
93. Jiao L, Mu ZG, Zhu CZ et al (2016) Graphene loaded bimetallic Au@Pt nanodendrites enhancing ultrasensitive electrochemical immunoassay of AFP. Sens Actuat B Chem 231:513–519. https://doi.org/10.1016/j.snb.2016.03.034
94. Lim SA, Yoshikawa H, Tamiya E et al (2014) A highly sensitive gold nanoparticle bioprobe based electrochemical immunosensor using screen printed graphene biochip. RSC Adv 4(102):58460–58466. https://doi.org/10.1039/C4RA11066H
95. Liu GD, Lin YY, Wang J et al (2007) Disposable electrochemical immunosensor diagnosis device based on nanoparticle probe and immunochromatographic strip. Anal Chem 79(20):7644–7653. https://doi.org/10.1021/ac070691i
96. Ricci F, Adornetto G, Palleschi G (2012) A review of experimental aspects of electrochemical immunosensors. Electrochim Acta 84:74–83. https://doi.org/10.1016/j.electacta.2012.06.033
97. Dutta G, Lillehoj PB (2018) Wash-free, label-free immunoassay for rapid electrochemical detection of *Pf*HRP2 in whole blood samples. Sci Rep 8(1):17129. https://doi.org/10.1038/s41598-018-35471-8
98. Tuteja SK, Ormsby C, Neethirajan S (2018) Noninvasive label-free detection of cortisol and lactate using graphene embedded screen-printed electrode. Nanomicro Lett 10(3):41. https://doi.org/10.1007/s40820-018-0193-5
99. Khan MS, Dighe K, Wang Z et al (2019) Electrochemical-digital immunosensor with enhanced sensitivity for detecting human salivary glucocorticoid hormone. Analyst 144(4):1448–1457. https://doi.org/10.1039/C8AN02085J
100. Kamakoti V, Selvam AP, Shanmugam NR et al (2016) Flexible molybdenum electrodes towards designing affinity based protein biosensors. Biosensors 6(3):36. https://doi.org/10.3390/bios6030036
101. Mehlhorn A, Rahimi P, Joseph Y (2018) Aptamer-based biosensors for antibiotic detection: a review. Biosensors 8(2):54. https://doi.org/10.3390/bios8020054
102. Sypabekova M, Jolly P, Estrela P et al (2019) Electrochemical aptasensor using optimized surface chemistry for the detection of *Mycobacterium tuberculosis* secreted protein MPT64 in human serum. Biosens Bioelectron 123:141–151. https://doi.org/10.1016/j.bios.2018.07.053
103. Lin KC, Jagannath B, Muthukumar S et al (2017) Sub-picomolar label-free detection of thrombin using electrochemical impedance spectroscopy of aptamer-functionalized MoS_2. Analyst 142(15):2770–2780. https://doi.org/10.1039/C7AN00548B
104. Xue J, Li Y, Liu J et al (2022) Highly sensitive electrochemical aptasensor for SARS-CoV-2 antigen detection based on aptamer-binding induced multiple hairpin assembly signal amplification. Talanta 248:123605. https://doi.org/10.1016/j.talanta.2022.123605
105. Yarahmadi S, Azadbakht A, Derikvand RM (2019) Hybrid synthetic receptor composed of molecularly imprinted polydopamine and aptamers for impedimetric biosensing of urea. Mikrochim Acta 186(2):71. https://doi.org/10.1007/s00604-018-3180-0
106. Li Y, Li X, Dong CK et al (2010) A graphene oxide-based molecularly imprinted polymer platform for detecting endocrine disrupting chemicals. Carbon 48(12):3427–3433. https://doi.org/10.1016/j.carbon.2010.05.038
107. Farid MM, Goudini L, Piri F et al (2016) Molecular imprinting method for fabricating novel glucose sensor: polyvinyl acetate electrode reinforced by MnO_2/CuO loaded on graphene oxide nanoparticles. Food Chem 194:61–67. https://doi.org/10.1016/j.foodchem.2015.07.128