

Flexible Electrochemical Bioelectronics: The Rise of In Situ Bioanalysis

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The amalgamation of flexible electronics in biological systems has shaped the way health and medicine are administered. The growing field of flexible electrochemical bioelectronics enables the in situ quantification of a variety of chemical constituents present in the human body and holds great promise for personalized health monitoring owing to its unique advantages such as inherent wearability, high sensitivity, high selectivity, and low cost. It represents a promising alternative to probe biomarkers in the human body in a simpler method compared to conventional instrumental analytical techniques. Various bioanalytical technologies are employed in flexible electrochemical bioelectronics, including ion-selective potentiometry, enzymatic amperometry, potential sweep voltammetry, field-effect transistors, affinity-based biosensing, as well as biofuel cells. Recent key innovations in flexible electrochemical bioelectronics from electrochemical sensing modalities, materials, systems, fabrication, to applications are summarized and highlighted. The challenges and opportunities in this field moving forward toward future preventive and personalized medicine devices are also discussed.

flexible materials into bioelectronics for a suitable configuration in healthcare applications.^[6–9] Because of the unique advantages such as light weight, high flexibility, and great conformability, flexible bioelectronics allows natural interaction between electronics and the soft human body, and enables real-time and continuous physiological and biological monitoring.^[10–13] Today, flexible bioelectronics are prominently found in wearable electronics with integrated biosensors embedded in a smartwatch or a smartphone.^[14] They facilitate continuous monitoring of the physiological status of individuals and provide a personalized health profile to keep track of an individual's well-being.^[15,16] Over the last decade, flexible and stretchable bioelectronics have further spearheaded the research development in biological sensing platforms and point-of-care diagnostic devices.^[17–20] They allow

continuous assessment of electrophysiological, physiological, mechanical and biochemical information from the human body.^[21–24] Yet today's market for wearable electronics is limited to tracking physical and vital signs. Incorporation of bioelectronics that is informative at biomolecular level opens up opportunities to promote health management.^[25,26] To this end, flexible electrochemical bioelectronics devices are most attractive in situ molecular monitoring approach owing their unique advantages in inherent wearability/portability, high sensitivity, high selectivity, and low cost.^[27–29]

Flexible electrochemical bioelectronics aims to tackle human biomolecular information by utilization of biological elements in electrochemical reactions.^[30,31] It leverages the advances in microelectronics and materials technology, analytical electrochemistry, together with the growing understanding of human biology and biomedical research.^[32,33] A typical electrochemical bioelectronic system is composed of three major components: a biorecognition element, an electrochemical signal transducer, and a signal acquisition module. The biorecognition element interacts with the target analyte to exchange or transfer ions and electrons such that the concentration of target analyte can be quantified. The electrochemical signal transducer converts such interaction into a measurable format such as electrical current and potential. A signal acquisition system is an analytical device that allows data acquisition, processing, analysis, and display in user-friendly configuration. Depending on the sensing modalities and target applications, it may contain simple electronic components with signal processors or advanced electronic

1. Introduction

Integration of electronic systems and bioengineering is a rapidly growing research area driven by its promising potential to assist fundamental research, health and environmental managements.^[1–5] Together with the growing interest in bioelectronics, flexible technology introduces novel devices by merging

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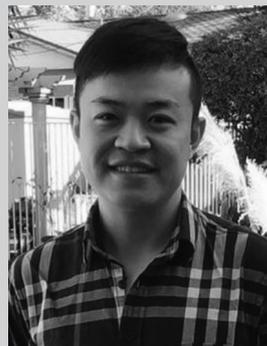
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circuitry to support the biosensing measurement. The rapid advancement of engineering biomaterials provides novel means to synthesize biosubstances such as enzymes, protein receptors, and aptamers, and they serve as functional units for emergence with electrical elements toward in situ electrochemical biosensing. Major efforts in flexible electrochemical bioelectronics advocate on biosensors and biofuel cells, arising from biomolecular detection schemes.^[34] Flexible biosensors allow the in situ quantification of chemical constituents present in the human body. This represents a promising alternative to probe biomarkers in the human body in a simpler method compared to conventional instrumental analytical techniques. Recent developments in biosensors have targeted detection of analytes in a wide range of biofluids especially in blood, cerebrospinal fluid, interstitial fluid, sweat, saliva, and tears (**Figure 1**). Biofuel cells exploit such method to take a step further to power simple electronic devices or to function as self-powered biosensors.

To understand recent research development of flexible electrochemical bioelectronics, it is paramount to realize current target biological elements and their importance in biomedical research. Here we briefly introduce emerging biofluids being exploited in bioelectronics. Blood has traditionally been the most powerful tool to retrieve information to physiological functions, medical diagnosis, and therapy. Cerebrospinal fluid (CSF) contains a number of informative analytes for neurological monitoring and investigation. Both blood and CSF are sampled invasively by venipuncture or accessed via implantable devices. Due to its invasive procedures, there is a rapidly growing interest in alternative biofluids for noninvasive assessment. Interstitial fluid that bathes microenvironment of cells serves as the transport medium for nutrients, signaling substances, and wastes between cells and the blood capillaries.^[35] It contains chemicals in similar concentrations as blood with less content of proteins. It can be extracted invasively with microneedles or transdermally through near-infrared spectroscopy, ultrasound and reverse iontophoresis, hence offering a promising target for noninvasive health assessment. Currently, interstitial fluid is most commonly used in continuous monitoring of blood glucose levels for its glucose correlation with blood glucose concentrations. Saliva, an ultrafiltrate from plasma, is another attractive sample since it can be collected in larger quantity and is being used in metabolomics.^[36] For instance, salivary and blood ethanol had been found to be well correlated.^[37] Tear is another alternative and contains a large number of proteins, along with salt, metabolites, and immunoglobulin, to maintain a healthy ocular surface.^[38] Due to its rich chemical compositions, noninvasive tear analysis is another rising research area though it is not as well developed due to its sophisticated requirement on electronics for its wearability. Lastly, sweat is an emerging noninvasive biofluid that is readily accessible by physical exercises, thermal exertion, and chemical induction called iontophoresis. It is composed of ions, metabolites, amino acids, and proteins but mostly at a trace level. Its ease of access thrives the development of wearable bioelectronics for sweat sensing at a fast pace in recent years.

Here, we aim to present recent key innovations in flexible electrochemical bioelectronics which are setting the basis for future preventive and personalized medicine devices and approaches. In the following sections, we describe in details



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the major electrochemical sensing modalities, materials, and applications in the flexible bioelectronics. We review the bioanalytical technologies employed in flexible electrochemical bioelectronics, including ion-selective potentiometry, enzymatic amperometry, potential sweep voltammetry, field-effect transistors, affinity-based biosensing, and biofuel cells. Recent advances on the low-cost fabrication of such flexible electrochemical bioelectronics are also described.

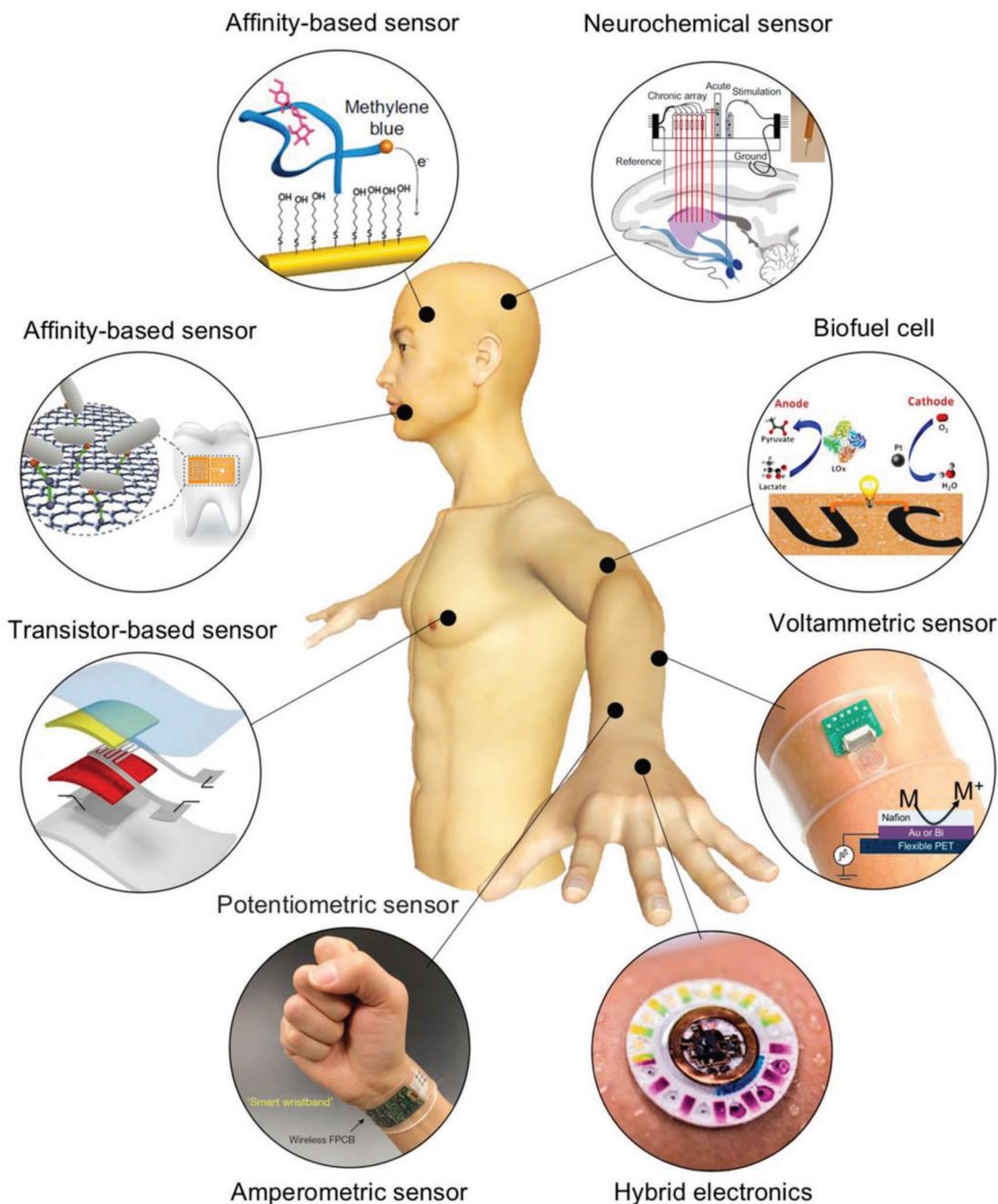


Figure 1. Overview of the flexible electrochemical bioelectronics. Potentiometric sensor and amperometric sensor image: Reproduced with permission.^[46] Copyright 2016, Springer Nature. Transistor-based sensor image: Reproduced with permission.^[162] Copyright 2014, Wiley-VCH. Affinity-based sensor image: Reproduced with permission.^[151] Copyright 2012, Springer Nature. Affinity-based sensor image: Reproduced with permission.^[158] Copyright 2017, National Academy of Sciences. Neurochemical sensor image: Reproduced with permission.^[129] Copyright 2017, National Academy of Sciences. Biofuel cell image: Reproduced with permission.^[182] Copyright 2013, Wiley-VCH. Voltammetric sensor image: Reproduced with permission.^[133] Copyright 2016, American Chemical Society. Hybrid electronics image: Reproduced with permission.^[96] Copyright 2019, American Association for the Advancement of Science. Reprinted/adapted from ref. [96]. © The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. Distributed under a Creative Commons Attribution NonCommercial License 4.0 (CC BY-NC) <http://creativecommons.org/licenses/by-nc/4.0/>.

2. Flexible Potentiometric Sensors

Potentiometric sensors measure the open circuit electrical potential difference between the sensing electrode and the reference electrode. This type of sensors typically employs a

two-electrode system in which the sensing electrode is functionalized with a target recognition element and the reference electrode is formulated to achieve a stable electrical potential. The response of these sensors is governed by the Nernst equation, which describes that the potential at the sensing electrode

is altered by 59.2 mV per unit charge at 25 °C upon a decade change in target analyte concentration.^[39] Wearable potentiometric sensors integrate these sensors into a conformal platform to achieve coherent structure and easy wearability depending on applications. They have been widely investigated for detection of ions,^[40–42] drugs,^[43,44] organic compounds,^[45] etc. Currently, the most common wearable potentiometric sensors are ion-selective sensors for detection of small ions such as Na⁺, K⁺, and H⁺.^[46–52] They convert the input ionic signal to measurable electronic signal. These sensors have one of the simplest forms of sensing modality as the response is determined by ion exchange/transfer processes at the selective membrane–liquid interface. With major developments of sensing materials chemistry, sensitive and selective sensors can be quickly achieved for ionic detection in biofluids. However, many limitations remain to be addressed to meet the requirements of integration onto flexible electronics. Due to the nature of biofluids and point-of-care devices, it is necessary that sensors can accurately measure compositions at small volumes and have minimal calibration with fast response time, long-term stability, extended lifetime, and durability.

2.1. Materials for Ion-Selective Sensors

Conventional ion-selective electrodes require an inner filling solution with a high concentration of target analyte such that the potential difference across the selective membrane between the inner and test solutions can be measured. However, they are not compatible with wearable applications as they contain liquid phase, have shape constraint, and are not easily miniaturizable.^[53] The ion-selective sensors being exploited in current flexible technology are all-solid-state sensors. These sensors contain a solid contact, which acts as an ion-to-electron signal transduction pathway, between the conductive electrode and the selective membrane layer(s) to enhance the stability of the sensor's signals. The selective membrane typically consists of ion-selective ionophores dissolved in complex polymeric matrix with high mechanical stability. Each of these layers is critical to produce high-performance ion-selective electrodes for long-term usage in wearable devices. On the other hand, reference electrodes are based on Ag/AgCl coated with a polymer matrix containing saturate Cl ions to maintain a stable potential.^[54]

A good solid contact is critical for correctly converting signals for accurate ionic detection and for achieving stable sensors. The solid contact used in ion-selective sensors should: 1) well adhere to conductive electrode, 2) have no chemical reactions with species present in the selective membrane and the test solution, 3) very lipophilic to prevent water layer formation between the selective membrane and the solid contact or very hydrophilic to act as an inner liquid filled reservoir, and 4) have high redox/double layer capacitance to achieve a stable potential quickly.^[55,56] Recent development of potentiometric sensors attempts to meet these requirements to achieve highly stable for continuous and long-term usage. The solid contact is typically made of conducting polymers, redox active self-assembled monolayers (SAMs), and nanomaterials with high capacitance.^[57–62] Conducting polymers are suitable candidates for solid contact as they have both ionic and electrical conductivity and convert ionic to electrical signal by doping–dedoping of

the conducting polymer.^[57] They can easily be drop-cast or electrodeposited on a conductive substrate. Most commonly used conducting polymers contain poly(3,4-ethylenedioxythiophene) doped with polystyrene sulfonate (PEDOT:PSS), polypyrrole, poly(3-octylthiophene) (POT), and polyaniline (PANI). They are electropolymerized on a conductive electrode by scanning cyclic voltammetry (CV), applying constant potential or current to achieve strong adhesion with uniform thickness. Conductive polymers like PANI, for instance, can act as a standalone pH sensor since it undergoes protonation–deprotonation and shows a Nernstian behavior to pH. Hence, they can interfere with the measured signal when the test solution has a change in pH. PEDOT:PSS is the most suitable conducting polymer to act as an ion-to-electron transducer due to its low sensitivity to O₂ and pH.^[63] In addition to conducting polymer, lipophilic SAMs such as tetrathiafulvalene (TTF), ferrocene derivatives, as well as redox couples are also exploited.^[64,65] SAMs can be deposited by simply dipping the conductive electrode into desired containing solution. Due to their lipophilicity, they minimize the formation of water layer and promote better adhesion between the SAMs and the selective membrane. It has been shown that this approach can lead to sensor variation of less than 1 mV.^[66] The fullerene and TTF derivative attached K⁺ sensors can detect down to 10×10^{-6} M [K⁺] with a drift of 85 μ V h⁻¹ for over 100 h.^[66] Another approach is increasing the interfacial area between the conductive electrode and the selective membrane to improve double layer capacitance. This can be achieved by electrochemical deposition of inert nanomaterials/features such as gold, platinum, carbon-based nanofeatures.^[67–69] Such approach allows sensor to maintain its performance during long-term storage with a signal drift as small as 6.3 μ V h⁻¹ in 172 h continuous measurement.^[69]

The selective membrane layer decides the sensitivity and selectivity of the ion-selective sensors. The membrane layer is typically composed of lipophilic ion exchanger, ionophore, plasticizer, and polymer matrix dissolved in organic solvent.^[70] Together, they serve to provide optimal sensors performance although much effort is still needed for continuous measurement in wearables. Major challenges with the membrane layer include sensor degradation due to chemical leaching and water layer formation. Most commonly PVC is used as a polymer matrix, together with plasticizer in wearable ion-selective sensors to achieve mechanically stable and well adhesive membrane with the substrate. Other polymers such as polyacrylates and semi-fluorinated polymers are less common and have also been shown as promising polymer matrixes to support ion-selective membrane.^[71] It has been shown that addition of polyurethane and silicone rubber increases the hydrophobicity of the membrane layer, and thus enhances the sensor stability and lifetime.^[62,72] Incorporation of nanomaterials such as CNTs has also presented to further improve the stability of the sensor due to its hydrophobic nature.^[73] However, potential toxicity of leaching chemicals limits utilization of nanomaterials inside membrane layer.

2.2. Flexible Ion-Selective Sensors

Monitoring electrolytes balance in biofluids is critical for its role in maintaining healthy metabolic functions and nerve activities.

Among various ions found in human body fluids, detection of Na ions has been the most prominent in wearable research due to its presence at high concentration in biofluids and physiological importance for maintaining fluid and electrolyte balance. **Figure 2a** shows an example of a wearable ion-selective sensor for continuous monitoring of sweat ions. The electrodes were directly screen-printed on an adhesive bandage, and the selective layer (polyaniline in this case) was electrochemically deposited such that printed electrodes side could adhere and interface with the skin for *in vivo* detection of sweat pH.^[74] Alternatives to patterning electrodes on flexible substrates (e.g., tattoo transfer adhesive,^[49] textile,^[75] and polyethylene terephthalate^[52]), conductive wires such as platinum, silver, and carbon fibers^[51,76] could also be directly functionalized into ion-selective sensors and are inserted inside skin conformal substrate for sweat monitoring. The limitations of such simple design include trapping sweat and its contents between the

sensor and the skin, introducing possible blocked glands and measurement artifacts. One method to overcome early challenge is introducing porous features in cellular network format as shown in Figure 2b.^[77] The presence of pores facilitates sweat flow with enhanced breathability for sweat glands while minimizing sweat artifacts due to sweat accumulation underneath the sensor. Another approach is incorporation of wicking fabric near the sensing site to remove old sweat accumulation such that sensor readings can be up-to-date.^[78] Electrochemical sweat sensor incorporated with microfluidics is also a promising alternative to achieve efficient sweat sampling and minimize sweat artifacts. The advantages of microfluidics are that they allow sweat flow from secretion sites to directed micro-channel for effective sweat sampling to provide real-time sweat ionic levels. These sensors utilize sweat secretion pressure and capillary action to support sweat flow such that priorly secreted sweat is guided through the microfluidic, and sensors can

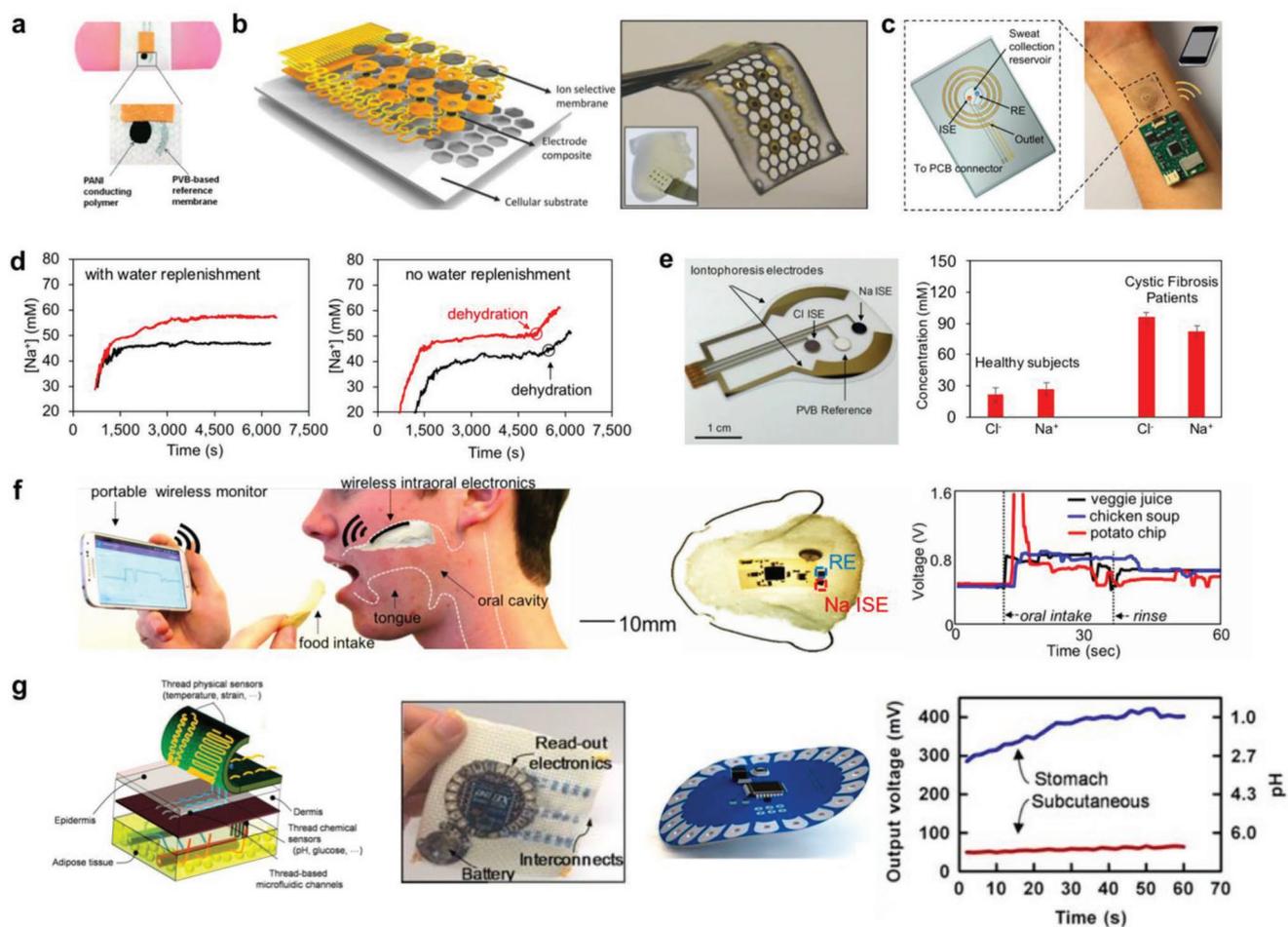


Figure 2. Flexible potentiometric ion-selective sensors for detection of ions in biofluids. a) A band-aid style sweat pH sensor. Reproduced with permission.^[74] Copyright 2014, Wiley-VCH. b) A porous cellular network for detection of Na. Reproduced with permission.^[77] Copyright 2017, Wiley-VCH. c) A microfluidic patch for simultaneous monitoring of sweat ions and secretion rate. Reproduced with permission.^[80] Copyright 2018, American Chemical Society. d) Real-time monitoring of sweat Na⁺ during the outdoor running of two healthy subjects with and without water replenishment. Reproduced with permission.^[46] Copyright 2016, Springer Nature. e) An autonomous sweat extraction and sensing of Na⁺ and Cl⁻ for diagnosis of cystic fibrosis. Reproduced with permission.^[82] Copyright 2017, National Academy of Sciences. f) An oral retainer embedded with a Na⁺ sensor and electronics for monitoring Na⁺ intake (shown in terms of voltage). Reproduced with permission.^[83] Copyright 2018, National Academy of Sciences. g) A thread-based pH sensor integrated with electronics for invasive subcutaneous and gastric pH monitoring. Reproduced under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>).^[84] Copyright 2016, The Authors, published by Springer Nature.

measure freshly secreted sweat at a specific time interval. The ions such as Na^+ and K^+ could be detected in microfluidic collection chamber with microfluidic routed channels.^[79,80] Interestingly, the device shown in Figure 2c allows simultaneous detection of sweat rate based on the change in impedance between two parallel metal electrodes. Incorporation of sweat rate monitoring further assists in understanding inter-related sweat parameters due to secretion rate.

To make it into wearable, flexible sensors are interfaced with electronic components to achieve an integrated platform for direct measurement of sweat compositions without external circuitry. Devices in Figure 2c, for instance, allow a printed circuit board (PCB) to interface with the sensing components containing signal conditioning, analysis, and wireless transmission to external cellphone or computer. Alternately, radio frequency has also been exploited for data transmission to a cellphone when the device containing RFID tags is in proximity to the RFID reader.^[81] These types of sensors can be attached onto the skin at various locations. A strap format with flexible ion-selective sensor and transistor, integrated with electronics for wireless continuous monitoring of Na^+ in sweat during intense exercise, was also been demonstrated. It allows tight and robust placement of sensors such as chest-strap heart rate monitors. With the development of wearable sensors, it becomes utmost important to show its applications in health monitoring. As exhibited in Figure 2d, sweat Na^+ increases abruptly after approximately 2 h of outdoor running when there is no replenishment for lost water, making sweat Na^+ a potential biomarker for hydration status.^[46] Considering that the sweat Cl^- has been the gold standard for cystic fibrosis diagnosis, researchers developed a skin-worn wearable platform for local sweat extraction and sweat Na^+ and Cl^- determination using flexible ion-selective sensors (Figure 2e). Real-time study results obtained from healthy and cystic fibrosis patients using this device was demonstrated, indicating the promise of using wearable potentiometric sensors toward rapid cystic fibrosis screening.^[82] In another case, real-time sodium intake was monitored using wireless intraoral electronics contained in a dental retainer (Figure 2f). The sensor together with electronic circuits was embedded in an elastomeric membrane and allowed long-range wireless communication for real-time tracking of saliva sodium concentration.^[83] Besides, ion sensing was also studied in gastric and subcutaneous fluids. Specifically, Figure 2g represents thread-based pH sensor integrated with electronic circuits, fabricated on woven fabrics, for independent monitoring of real-time change in stomach acidity.^[84] All of these devices allow wireless transmission of data for real-time readout of ion concentrations.

3. Flexible Amperometric Sensors Based on Enzymatic Electrodes

Amperometric sensors measure current signals produced by redox reactions at the electrode–liquid interface under an applied potential. Concentration of target analytes is deduced from the relation between the intensity of the current to concentration of the target. For dynamic quantification, this current is monitored with respect to time and is converted to respective

concentration, making amperometric sensors appealing for monitoring target analytes in flow systems. This type of sensors commonly utilizes three-electrode system, which contains a working electrode that is functionalized with a target recognition element, a counter electrode for the current source, and a reference electrode with a stable potential. Amperometric sensors can achieve low detection limit with high selectivity in a small sample volume, making them an ideal candidate for developing flexible and wearable devices toward in situ and continuous health monitoring.^[39] The most common type of sensors is enzymatic sensors, in which target specific enzymes are functionalized on the working electrode to catalyze the chemical reaction with the target. It is a widely utilized detection method in wearable electronics for sensing metabolites for its simplicity in fabrication as well as measurement and data collection. In the following sections, we review the current technology of enzyme-based amperometric sensors in wearable bioelectronics and its usage in study of compositions of biofluids.

Most of the enzymatic sensors utilized in wearable devices use oxidases, which ensures high selectivity of the sensors.^[85] For instance, glucose oxidase is a well-known biorecognition element for reaction with glucose and production of H_2O_2 under the presence of O_2 . The product H_2O_2 , in turn is further reduced by a redox mediator (e.g., Prussian blue) to eventually quantify glucose concentration.^[86] Prussian blue is a widely used mediator because it readily reduces H_2O_2 at a low redox potential and is an active electrocatalyst for H_2O_2 compared to the traditional electrodes such as gold and platinum. Other mediators such as ferricyanide, ferrocene, and methylene blue have also been shown as effective mediators for assisting electron transfer.^[85,87] Similarly, lactic acid, uric acid, and ethanol can be monitored via enzymatic sensors based on lactate oxidase, uricase, and alcohol oxidase, respectively.^[85,88] Nanomaterials such as ZnO , CdS , Pd , and Fe_3O_4 are also utilized to facilitate electron transfer and lower detection limit.^[89–91]

Despite these enzymatic sensors can achieve high performance in standard test solutions, biofluids require sensors to be extremely robust. Adsorption of small molecules and proteins in biofluids can degrade sensors rapidly by hindering electrocatalysis and target diffusion inside the membrane layer. Passivation of the electrode surface and polymeric films can also inhibit effective sensing capabilities, especially in the case of direct electron transfer.^[87] Biofouling and passivation can be minimized by applying a small voltage that causes redox reaction of the target but not the interfering species. To further reduce these issues, immobilization of enzymes in a polymer matrix and polymer crosslinking are commonly used strategies. Enzymes are immobilized by reorientation using PEI, covalent binding and physical entrapment in polymers such as Nafion, agarose, PVA, etc.^[87]

3.1. Flexible Enzymatic Sensors for Sweat Glucose Analysis

Glucose is one of the major metabolites secreted in sweat at a much lower concentration than in blood.^[92] It is the most prominent metabolic target in sweat for its potential to assist prediabetes monitoring and diabetes management in a noninvasive and continuous manner. To understand the role of sweat glucose in diabetes management, reliable wearable sweat glucose

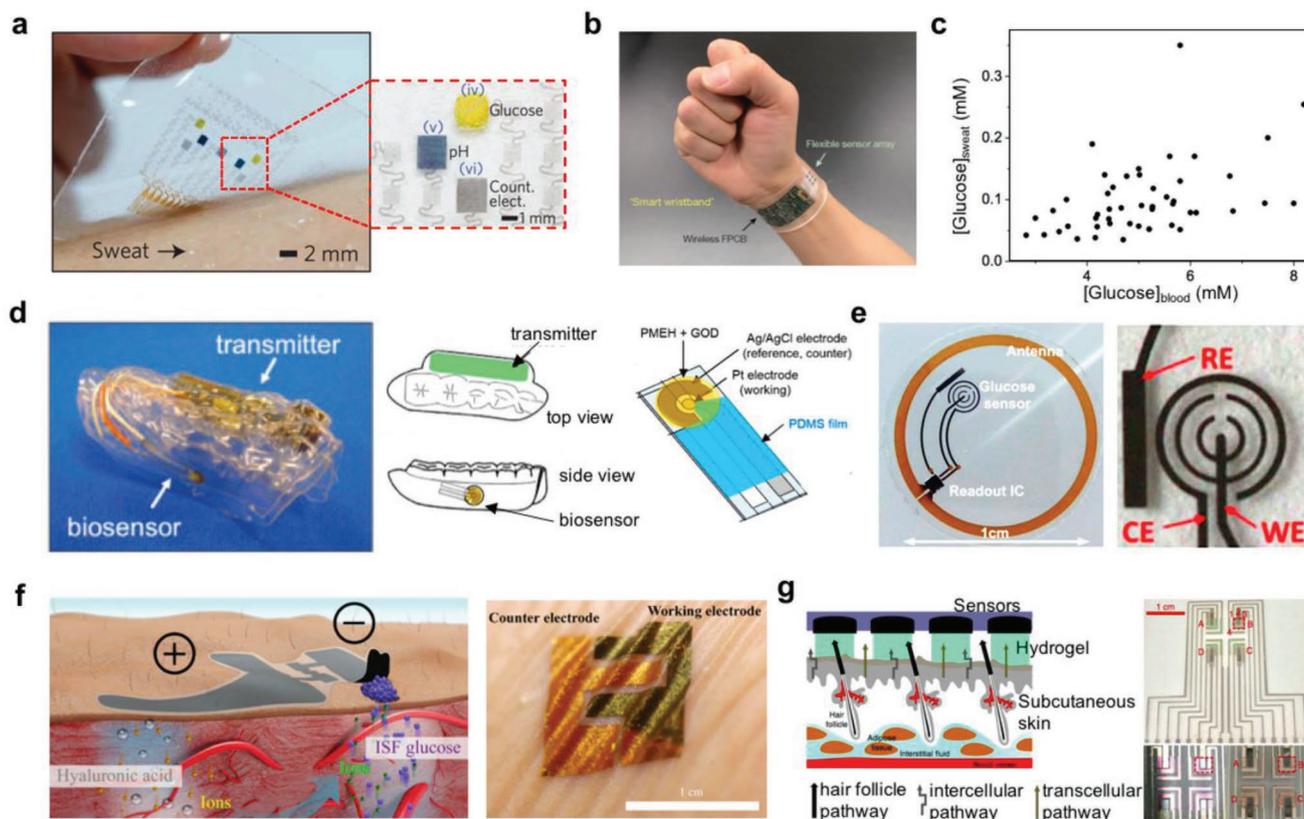


Figure 3. Flexible enzymatic sensors for glucose monitoring in biofluids. a) A patch style sweat sensor for continuous monitoring of glucose and pH levels. Reproduced with permission.^[93] Copyright 2016, Springer Nature. b) A smart wristband for simultaneous detection of sweat Na⁺, K⁺, glucose, lactate, and skin temperature. Reproduced with permission.^[46] Copyright 2016, Springer Nature. c) The relation between iontophoretic sweat and blood glucose levels in healthy subjects (sweat measurements were obtained from glucose sensor). Reproduced with permission.^[100] Copyright 2019, American Chemical Society. d) An oral retainer containing a glucose sensor and wireless transmitter for monitoring saliva glucose level. Reproduced with permission.^[103] Copyright 2016, Elsevier B.V. e) A contact lens embedded with tiny glucose sensors and IC chip for detection of tear glucose. Reproduced with permission.^[104] Copyright 2011, IEEE. f) A wearable interstitial fluid glucose extraction and detection device. Reproduced with permission.^[110] Copyright 2017, American Association for the Advancement of Science. Reprinted/adapted from ref. [101]. © The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. Distributed under a Creative Commons Attribution NonCommercial License 4.0 (CC BY-NC) <http://creativecommons.org/licenses/by-nc/4.0/>. g) A pixelated interstitial fluid glucose extraction and sensing platform. Reproduced with permission.^[111] Copyright 2018, Springer Nature.

sensors play a critical role to ensure measurement accuracy without sweat and sensing artifacts. There are several form factors proposed for sweat glucose monitoring. For instance, tattoo and patch style sensors (Figure 3a) allow intimate contact with the skin such that sweat glucose can be detected as soon as sweat secretes.^[93,94] Figure 3b shows a sensor packaged in wristband format. The sensing component in this wristband is disposable and can be replaced every time by insertion into the connector of the flexible electronic component.^[46] The presence of an absorbent pad between the sensing component and the skin allows sweat accumulation in the pad, eliminating direct contact and minimizing abrasion. Another is a strip-style disposable sensor which allows sweat uptake into the strip and detects glucose level in the strip by inserting to a separate smartband for independent analysis.^[95] This type of device separates sweat collection and sensing to minimize artifacts arisen from continuous movement and contact with the skin. It allows short-term usage of sensors to provide discrete-time dependent sweat glucose information. An alternative method is utilizing microfluidics for controlled sweat sampling and detection.^[96] The device incorporates near field communication

(NFC) for simplification of electronic circuitry. As enzymes can denature depending on operating temperature and pH, glucose sensors require careful calibration and compensation to achieve accurate measurement with minimal influence from external interferences. To tackle these problems, multiplexed sensors that integrate multiple modalities in a single device for simultaneous monitoring of glucose along with real-time pH and temperature calibration have also been demonstrated.^[46,97]

Sweat and blood glucose correlation study has been a popular research focus, and it remains unclear whether sweat and blood glucose have a good correlation based on earlier works on collected sweat.^[98,99] Wearable devices enable tracking glucose dynamics more closely to investigate sweat and blood glucose correlation. In a study for tracking iontophoretic sweat and blood glucose in seven healthy subjects, sweat glucose increased along with blood glucose in six subjects an hour after 30 g glucose intake.^[82] In a more comprehensive study conducted on 19 healthy subjects, subjects were given 75 g of oral glucose intake (Figure 3c), and a correlation coefficient of 0.44 was reported.^[100] Despite the weak correlation, it was also reported that normalized sweat and blood glucose

showed an improved correlation of 0.75. In this work, glucose sensor showed a stable signal (100% of initial response) for 25 h owing to the use of nickel hexacyanoferrate stabilized Prussian blue.^[100] In another study, sweat glucose was tracked for 14 h in two healthy volunteers, and sweat glucose closely tracked the blood glucose with a correlation coefficient of 0.83.^[101] In another study by the same group, a closer look of sweat glucose in relation to blood glucose in various type of exercise sweat was conducted on ten subjects. It was found that the correlation factors (sweat to blood glucose ratio) varied individually. By using individualized correlation factor, blood glucose could be predicted from sweat glucose, and a correlation coefficient of 0.86 was achieved.^[97] Yet larger scale studies with healthy and diabetic subjects are needed to present practicality of sweat glucose in estimating blood glucose noninvasively.

3.2. Flexible Enzymatic Sensors for Analyzing Glucose in Tear, Saliva, Interstitial Fluid, and Blood

Detection of glucose using a wearable platform has been also widely explored in other biofluids, such as saliva, tear, interstitial fluid, and blood. Saliva is a complex biofluid that secretes from parotid glands, and saliva glucose was reported to follow blood glucose closely.^[102] This offers a promising alternative to monitor blood glucose levels by tracking saliva and blood for each individual. In wearable technology, monitoring saliva glucose with oral platform assists real-time continuous assessment. It is also necessary that electronic components are integrated within the platform to allow remote monitoring. Figure 3d shows a detachable “Cavitas sensor” in which glucose sensing electrodes are attached to the mouthguard and a potentiostat with a wireless transmitter is encased on the side of the mouthguard.^[103] Despite the promising correlation between salivary and blood glucose, further exploration of saliva glucose detection is needed to establish its wearable application and large scale-correlation studies are necessary to confirm the role of saliva glucose for noninvasive prediction of blood glucose.

On the other hand, tear glucose sensing is more widespread. In quantification of tear glucose using wearable sensor, it is utmost important that the device does not cause eye irritation or hinder tear production. To this end, majority tear glucose sensors rely on optical detection. The most advanced form of tear glucose sensor, designed as a contact lens, was demonstrated in Figure 3e.^[104] The lens contains a glucose sensor fabricated on a sol–gel membrane, conventional material used in the contact lens, embedded with a tiny wireless chip, battery along with glucose sensor, and it can be powered at a distance of 15 cm. Contact lens is not the only method for tear glucose detection. Another platform proposed in 2014 contain a glucose sensor that could be worn under the eyelid.^[105] It utilized flexible coils shaped electrodes forming a helical chain, which was connected to ASIC including a potentiostat and transponder circuit. Although several works have shown that tear glucose has a close correlation with blood glucose, it is yet to confirm if these are influenced by collection method and other factors.^[106,107]

Correlation between interstitial and blood glucose concentrations has been well established. With the development of the implantable continuous glucose monitors (CGMs), interstitial

fluid (ISF) has been a popular target for continuous monitoring of glucose levels.^[108] Commercial products from Dexcom, Senseonics, and Abbott Laboratories offer minimally invasive ISF detection for weeks.^[109] Interstitial fluid glucose can also be noninvasively monitored by applying a small current through the skin, a process called reverse iontophoresis. GlucoWatch was developed based on this process but withdrawn from the market due to the resulted skin irritation.^[108] Recently, ISF glucose detection using the flexible epidermal sensor patches has been demonstrated (Figure 3f).^[94,110] Another type of sensing platform proposed is shown in Figure 3g. It contains pixelated electrodes to target hair follicles as extraction sites to attain calibration-free glucose detection.^[111]

Flexible device for monitoring glucose in blood is also studied. They typically utilize subcutaneous implantation of glucose sensor such as catheter-based sensor for continuous invasive monitoring of blood levels.^[112] A flexible spirally rolled glucose sensor for monitoring blood glucose concentration via microcatheter was proposed.^[112] The spiral-shaped sensor utilized Kapton film as a substrate with glucose sensors fabricated on the inner side of the structure. Due to its flexible nature, they could be inserted into microcatheter to invasively monitor glucose level. Continuous blood glucose monitoring was not largely explored due to its major challenge with achieving reliable sensors in aggressive blood environment.

3.3. Flexible Enzymatic Sensors for Analyzing Targets Other Than Glucose

Wearable amperometric sensors have also been widely explored for detection of other targets other than glucose, especially in sweat and saliva. Flexible amperometric sensors for lactate, alcohol, and uric acid have been demonstrated in various forms including mouthguard and sweat sensors. Specifically, sweat lactate, which is strongly related with muscle activity and exercise intensity,^[113] has been monitored using wearable sweat lactate sensors (Figure 4a,b).^[114] Therefore, investigation of sweat lactate provides promising athletic applications. Wearable tattoo sensor developed by Wang group shown in Figure 4c,d allowed real-time sweat extraction and sweat ethanol tracking with the assistance of an integrated iontophoresis module.^[115] A detailed study performed by Heikenfeld and co-workers showed that sweat ethanol tracked glucose with a delay of 19–35 min.^[116] In addition to sweat analytes, saliva biomarker such as uric acid has been monitored using a fully integrated mouthguard in healthy subject and in hyperuricemia patient (Figure 4e,f).^[117]

4. Flexible Potential Sweep Voltammetric Sensors

Besides chronoamperometry being commonly used in enzymatic electrodes, a number of other voltammetric methods including cyclic voltammetry, linear sweep voltammetry (LSV), and pulse voltammetry, have been widely employed in flexible bioelectronics.^[118,119] These methods utilize a time-dependent varying voltage with respect to the reference electrode and measure the current response between the working and the counter electrodes. When a voltage lower than the redox potential

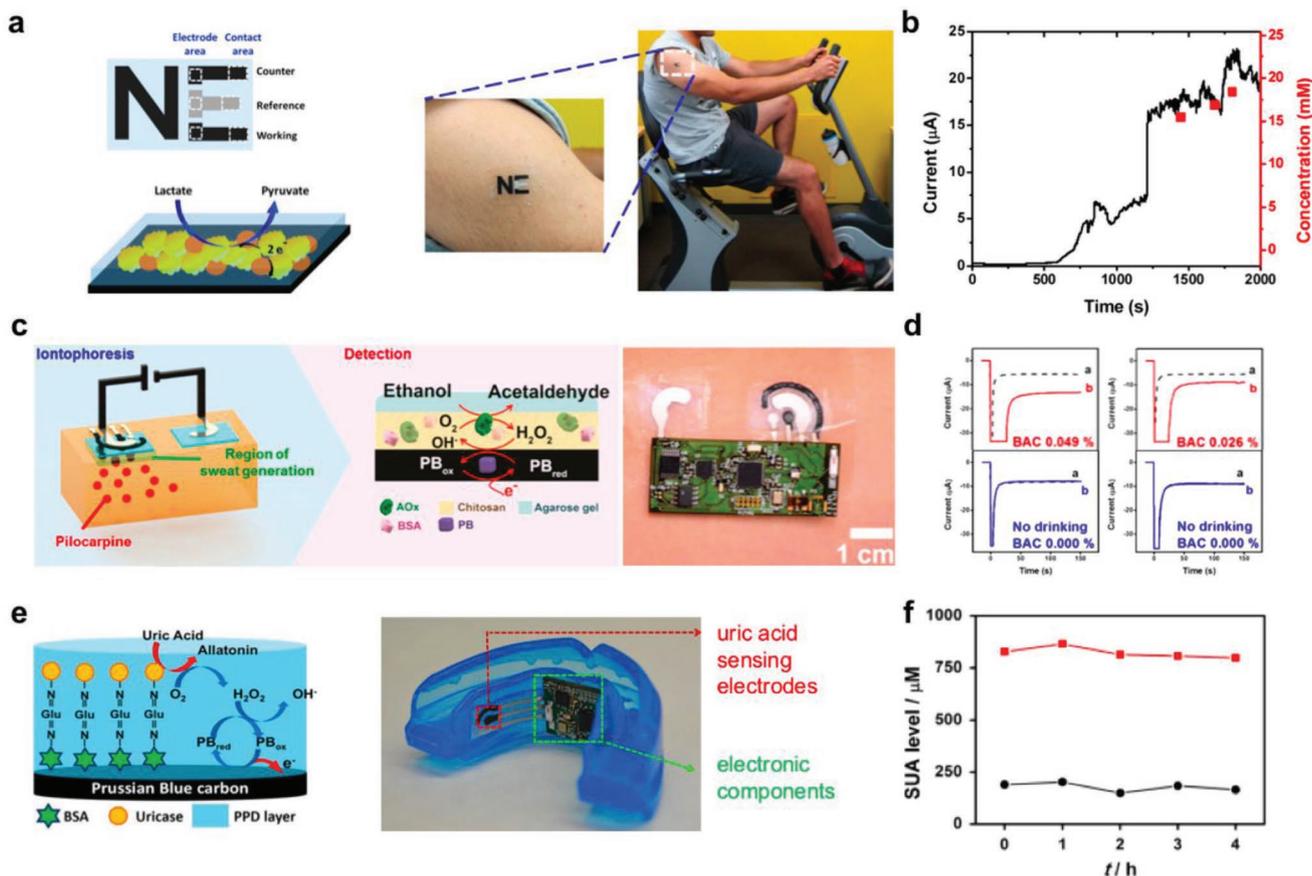


Figure 4. Flexible amperometric enzymatic biosensors for monitoring physiologically significant chemicals in biofluids. a) A wearable tattoo-based sweat lactate sensor. b) Continuous monitoring of sweat lactate during a cycling exercise. a,b) Reproduced with permission.^[114] Copyright 2013, American Chemical Society. c) A wearable tattoo-based alcohol sensor. d) Sweat alcohol monitoring using the wearable sensor from two subjects before and after consuming alcohol beverage. c,d) Reproduced with permission.^[115] Copyright 2016, American Chemical Society. e) A salivary uric acid sensing mouthguard. f) Salivary uric acid monitoring of a healthy volunteer (black) and a hyperuricemia patient (red) over a 5 h period. e,f) Reproduced with permission.^[117] Copyright 2015, Elsevier B.V.

of the electroactive species is applied, reduction occurs at the electrode–liquid interface. Similarly, oxidation occurs when the electrode potential is higher than the redox potential. Such voltammetric approach is an attractive solution for designing flexible bioelectronics toward monitoring a broad range of electroactive molecules in healthcare and security applications.

4.1. Flexible Sensors for Direct Detection of Electroactive Molecules

Unlike common metabolites (e.g., glucose or lactate) whose detection relies on the specific enzymes, electroactive molecules can directly lose or donate electrons on the electrode surface when a redox potential is applied to the sensing electrode.^[120,121] LSV and CV, which involve linearly stepped potentials, are usually employed as the first strategy to evaluate the electrochemical behavior of electroactive molecules on a specific working electrode due to the simple and rapid operation.^[122] However, in electrochemical detection, these methods result in low sensitivity due to a large background current arisen from charging at the electrode surface. To address this problem, differential pulse voltammetry (DPV) can be utilized

as an attractive approach to decrease the limit of detection. In DPV, the currents are recorded before the beginning and at the end of the potential pulse, and the current difference is monitored with respect to the voltage. Owing to the minimized effect of the charging current, high sensitivity down to nmol levels can be reached.^[123] Thus DPV could be used in flexible and wearable electronics to monitor ultralow levels of electroactive molecules in body fluids. As an example, a wearable sweat band for noninvasive and in situ methylxanthine drug monitoring was reported (Figure 5a).^[124] The fully integrated sweat band consists of a three-electrode system patterned on a flexible PET substrate and the electronic circuitry for signal conditioning, processing, and wireless transmission. Caffeine was chosen as the model drug and its electrochemical detection mechanism relied on the DPV, in which the oxidation of caffeine molecules at around 1.4 V was monitored (Figure 5b). The electrical current level detected at the oxidation peak provided a quantitative measurement of sweat caffeine concentrations. The use of carbon nanotubes (CNTs) and permselective Nafion coating significantly enhanced the sensitivity and long term stability during in situ sweat analysis. This wearable sensor was able to successfully detect few µmol level of caffeine in sweat from

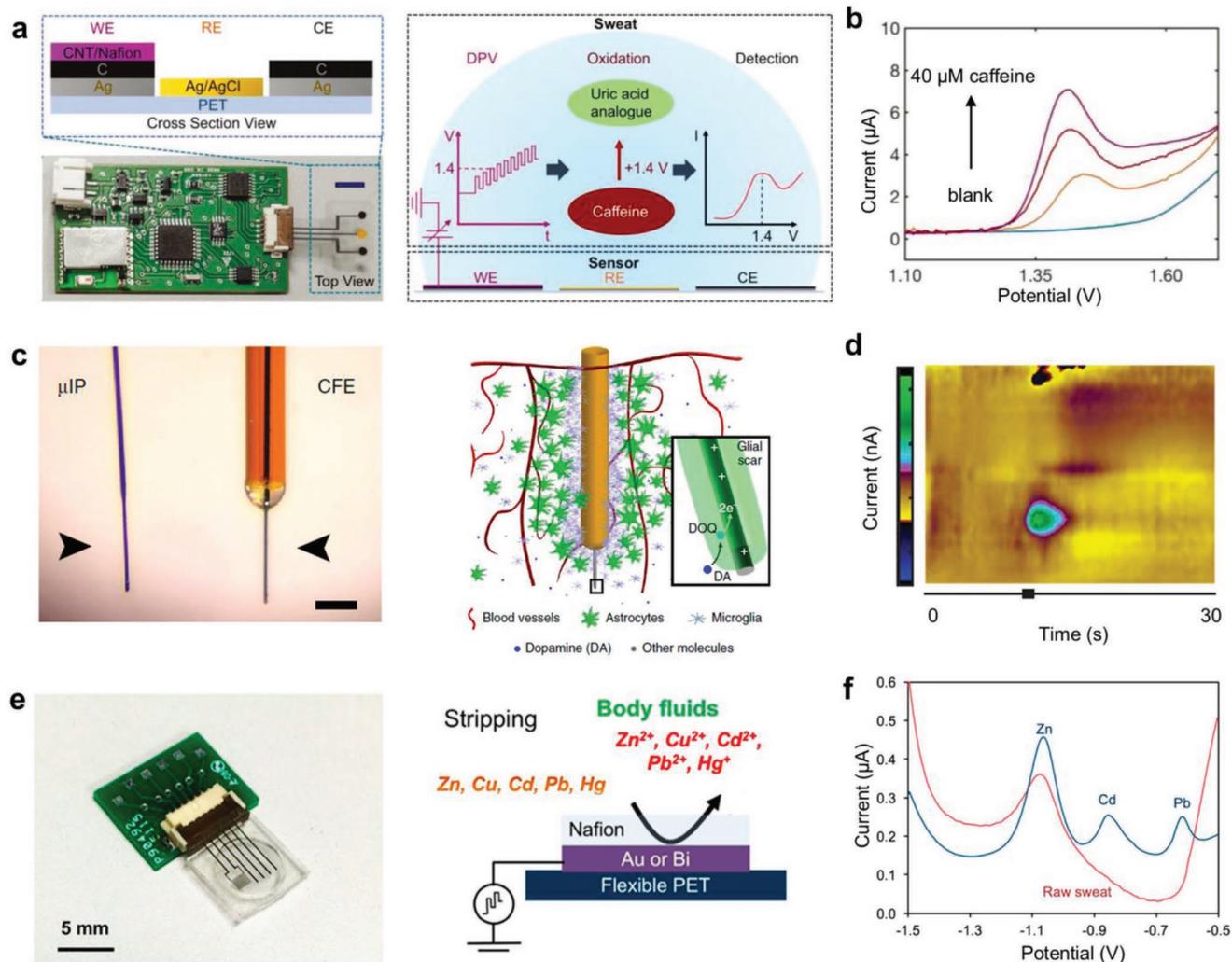


Figure 5. Flexible biosensors for continuous health monitoring using based on controlled potential voltammetry. a) Flexible sensor for continuous monitoring of ultralow levels of electroactive molecule—caffeine in human sweat using differential pulse voltammetry. Scale bar, 5 mm. b) DPV responses of a CNTs/Nafion-modified caffeine sensor in standard caffeine solutions. a,b) Reproduced with permission.^[124] Copyright 2018, Wiley-VCH. c) Flexible carbon fiber electrode for monitoring of neurotransmitter dopamine at high spatial and temporal resolution using fast scan cyclic voltammetry. Scale bar, 100 μm . d) Representative dopamine measurement from a chronically implanted probe in a rat. c,d) Reproduced under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>).^[130] Copyright 2018, The Authors, published by Springer Nature. e) A flexible microsensor array for sweat heavy metal ions monitoring via square-wave anodic stripping voltammetry. f) Multiplexed heavy metal analysis using the flexible sensor in collected human sweat. e,f) Reproduced with permission.^[133] Copyright 2016, American Chemical Society.

a healthy subject. Similar to DPV, other pulse voltammetric methods such as square-wave voltammetry (SWV) have been used as a sensing approach in flexible bioelectronics which can perform much faster measurement than DPV by applying higher scan rates up to 1 V s^{-1} . Flexible electronics coupled with pulse voltammetric sensing approach has shown broad applications beyond dynamic body fluid analysis. An integrated ring-based analytical platform for real-time detection of the surrounding chemical threats such as 2,4-dinitrotoluene (DNT) through the SWV was reported.^[125] Two well-defined peaks at -0.7 and -0.9 V were obtained which corresponded to the stepwise reduction of DNT. Additionally, a flexible glove-based biosensor based on SWV was developed toward monitoring of highly toxic organophosphate (OP) nerve-agent compounds.^[126] This platform utilized printed serpentine patterns to achieve

high stretchability. Organophosphorus hydrolase (OPH) enzyme was modified on the working electrode and the electroactive nitrophenol as the product of the enzymatic reaction was detected selectively via the SWV.

Although pulse voltammetry is among the most sensitive electrochemical sensing strategies, the in situ detection of neurotransmitters (such as dopamine, serotonin, noradrenaline, and adrenaline) requires high spatial resolution (micrometers) and high temporal resolution (milliseconds). To this end, fast scan cyclic voltammetry (FSCV) coupled with flexible carbon fiber electrodes is commonly utilized for continuous in vivo neurochemical sensing.^[127,128] In FSCV, a very high scan rate (up to $1 \times 10^6 \text{ V s}^{-1}$) is used to provide temporal resolution on a subsecond time scale. Due to such fast scan rate, the FSCV could also limit the diffusion distance of the charged molecules

to the electrode and minimize electrode fouling in vivo. During the redox reactions originated from the potential sweep, electrons are transferred between the analytes and the electrode, and the measured current is directly proportional to the number of molecules that undergo electro-oxidation. The plotted cyclic voltammogram provides chemical information that is unique for each analyte and thus allows identification of distinct analytes such as dopamine from other electroactive compounds. The detection of dopamine, a catecholamine neurotransmitter which plays several important roles such as transmitting signals in the brain and keeping the balance of metabolic functions in the body, has been intensively explored over the past decades for in vivo detection.^[129] As shown in Figure 5c,d, the carbon fiber-based microinvasive probes were able to stably monitor subsecond fluctuations of dopamine in anesthetized rats with nanomolar sensitivity over a 1 year period.^[130]

4.2. Flexible Sensors for Heavy Metal Detection via Stripping Voltammetry

In addition to direct detection of the electroactive molecules, voltammetric electrochemical sensors hold great promise for heavy metal monitoring in both biomedical and environmental settings.^[131] Heavy metals (e.g., Cu, Zn, Cd, Pb, and Hg) can be found in our body fluids such as blood, sweat, and urine. Excessive accumulation of heavy metals in the body can lead to chronic poisoning, Wilson's disease or even kidney and brain failure. The excretion of nonessential or toxic metals in sweat could be of toxicological and therapeutic importance for the early diagnosis. As reviewed earlier, the concentrations of metal ions (e.g., K^+ , Na^+ , and Ca^{2+}) could be potentially monitored via the solid-state ion-selective sensors. However, trace levels of heavy metal ions response (on the order of $\mu\text{g L}^{-1}$) can be hardly identified through potentiometric ion-selective sensor or direct electrochemical reduction from a single voltammetric scan. Thus, preaccumulation/deposition of the target ions is a necessary step to enable low concentration heavy metal sensing. In this regard, stripping voltammetry is an ideal electrochemical technique for highly sensitive multiplexed heavy metal analysis which involves three steps. First, heavy metal ions are electrochemically reduced onto the sensing electrode at a low potential. Second, the deposited heavy metal ions are stripped away or oxidized at specific potentials through DPV resulting in a current–voltage plot. Here, the current heights due to oxidation of heavy metals are used to quantify their concentrations. Lastly, a much higher potential is applied for a period of time to fully remove residual heavy metal ions from the electrode such that the electrode can be reused. Stripping voltammetry is usually based on a three-electrode system in which the working electrode materials play a crucial role in sensor performance. Although mercury drop electrode has been used for a number of stripping applications, it is not suitable for wearable sensing due to its toxicity and volatility. Alternative materials such as bismuth, gold, and carbon are stable and are more biocompatible, and hence they are more suitable for designing flexible biosensors toward continuous health monitoring. A previously reported wearable epidermal temporary tattoo sensor for zinc monitoring in human perspiration relied on an electroplated

bismuth film on a screen-printed carbon substrate.^[132] During the sensor process, Zn^{2+} was reduced to Zn^0 and preconcentrated on the sensing electrode at a -1.4 V first; the Zn film was then oxidized and quantified during the stripping voltammetry scan. From the zinc oxidation peaks at -1.15 V acquired at different time during the on-body trial, dynamic change in sweat zinc concentration was determined. Considering that our body fluids contain a variety of heavy metals, a flexible multiplexed microsensor array consisting of a Au and a Bi microelectrodes was developed on a flexible polyethylene terephthalate (PET) toward for multiplexed heavy metals analysis in body fluids (Figure 5e).^[133] The Au microelectrode offers excellent biocompatibility, a wide operational potential window owing to their high stability, and is ideally suitable for detection of Cu, Pb, and Hg. However, considering that the Zn stripping would result in strong hydrogen evolution on gold electrode due to the ultralow deposition potential, a Bi microelectrode was utilized instead for detecting Zn, Cd, and Pb. Bi electrodes offer great biocompatibility and high performance at low operation potentials (however, it is not suitable for stripping Cu and Hg due to its lower oxidation potential). Through the combination of both Au and Bi microelectrodes, this flexible platform could simultaneously monitor five heavy metals including Zn, Cd, Pb, Cu, and Hg. Each target could be qualitatively distinguished through the potential of the oxidation peaks. Real-time dynamic on-body monitoring of ultralow-level (a few hundred $\mu\text{g L}^{-1}$) sweat Zn and Cu was performed through a cycling exercise (Figure 5f).

Instead of directly using printed metallic or carbon electrodes, nanomaterials functionalized electrodes may be utilized to increase the electroactive area and the sensitivity of the direct electrochemical detection through pulse or stripping voltammetry. For example, a flexible sensor based on the micropatterned reduced graphene oxide (rGO) and carbon nanotube composite was reported to enable ultralow level detection of cadmium and lead ions.^[134] Porous laser-engraved graphene electrode on a flexible poly-L-cysteine substrate showed high sensitivity for the Pb^{2+} monitoring owing to the 3D porous interconnected electroactive graphene networks. The amino group of the L-cysteine could capture the heavy metal ions target due to the acid–base pairing interaction between electron-rich amino ligands and electron-deficient heavy metal ions and further enhance the sensing performance.^[135]

As we discussed above, a number of electroactive molecules and heavy metals could be readily detected through various controlled-potential voltammetries such as CV, LSV, DPV, SWV, and FSCV. Although these voltammetric approaches have distinct advantages on simplicity, sensitivity and low cost, major challenges on selectivity and biofouling need to be overcome in order to achieve continuous, stable, and selective measurement in vivo. To address these challenges, new nanomaterial innovations and antifouling strategies are strongly desired.

5. Flexible Field-Effect Transistors for Continuous Electrochemical Sensing

Flexible field-effect transistors (FETs) have recently received numerous attentions due to high off-state resistance, low power, low noise, large dynamic range, easy integration, and wide safe

operating range.^[136,137] A typical FET is a multilayer device, composed of source, drain, gate electrodes, and a dielectric layer that is interfaced with a semiconductor material.^[138] A large set of semiconductor materials, both organic or inorganic, can be used as an active channel in the flexible FET-based devices. In the case of the FET-based sensor, the specific interactions of the FETs with target charged biomolecules result in changes in the channel conductance and produce electronic current signals.

Enzyme-modified FETs have been explored toward continuous monitoring of the common metabolites including glucose, lactate, and uric acid.^[139–141] Flexible glucose sensing

FETs based on CVD grown graphene were demonstrated by functionalizing the enzyme GOx on graphene via the linker 1-pyrenebutanoic acid succinimidyl ester (PSE).^[142] Glucose detection (3.3–10.9 mmol) was obtained by direct measurement of the Dirac point shift and the drain-source current. As illustrated in **Figure 6a**, a flexible glucose sensor was developed based on In₂O₃ nanoribbon FETs with a fully integrated on-chip gold side gate.^[143] The In₂O₃ nanoribbon-based source and drain electrodes were modified with the GOx-loaded chitosan/CNT matrix to achieve a wide detection range (5 orders of magnitude) and an ultralow detection limit (10 nmol). The

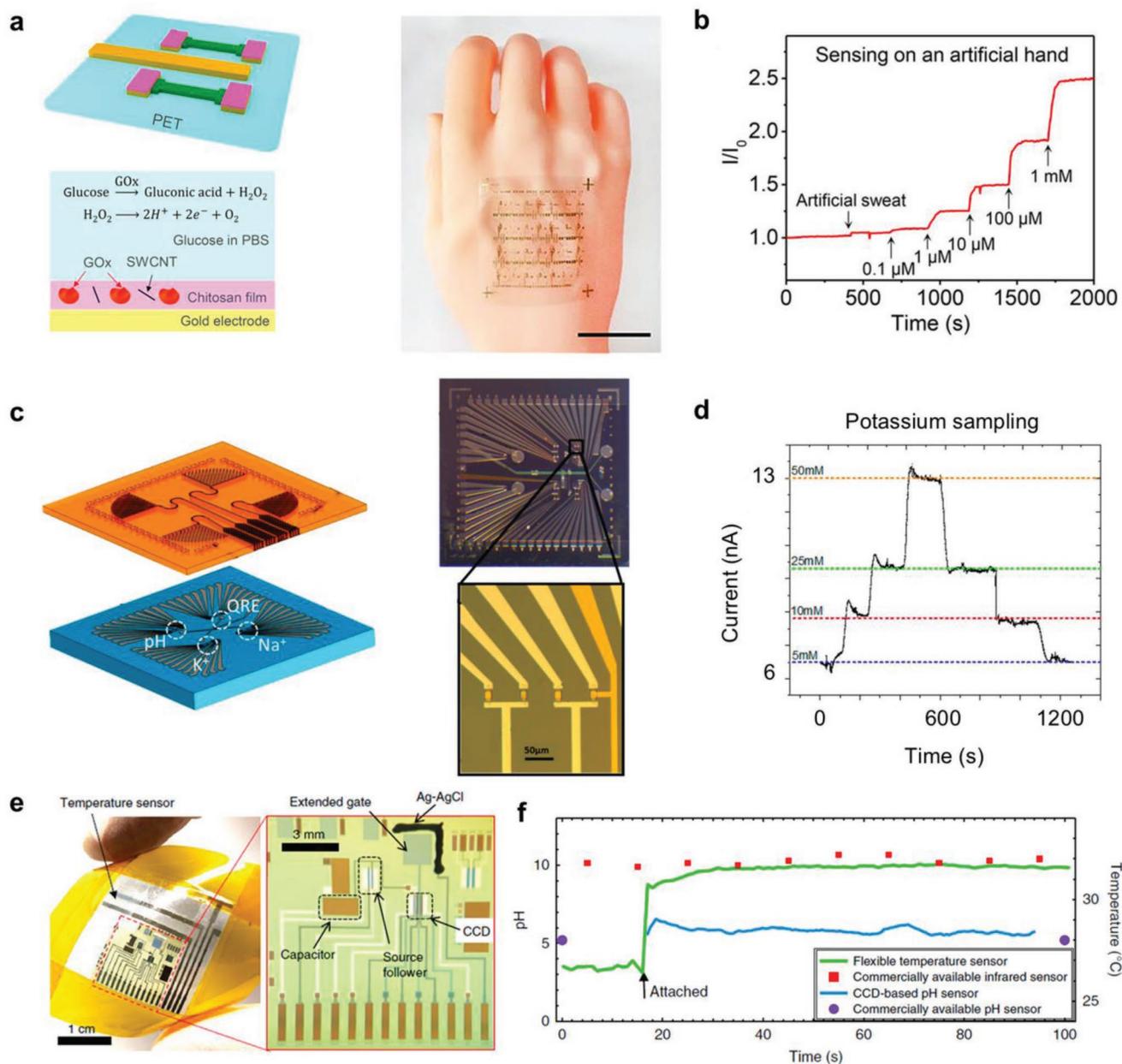


Figure 6. Flexible biosensors based on field-effect transistors. a) Enzyme-modified In₂O₃ FETs for continuous glucose monitoring. Scale bar, 3 cm. b) Real-time glucose-sensing results on an artificial arm using the In₂O₃ biosensor. a,b) Reproduced with permission.^[143] Copyright 2018, American Chemical Society. c) Ultralow-volume microfluidic device with ion-sensitive FETs. d) Real-time measurement of [K⁺] using an ion-selective FET. c,d) Reproduced with permission.^[147] Copyright 2018, American Chemical Society. e) A flexible charge-coupled FET-based wearable pH sensor integrated with a temperature sensor. f) Real-time in situ pH sensing using the charge-coupled FET-based pH sensor. e,f) Reproduced with permission.^[148] Copyright 2018, Springer Nature.

matrix accepts electrons in the presence of glucose and thereafter transfers electrons to molecular oxygen, consequently producing H_2O_2 . Further oxidation of H_2O_2 under a bias voltage resulted in a decreased pH, protonation of the OH groups on the In_2O_3 surface, and thus changes in current of the FETs (Figure 6b). The glucose sensing was also demonstrated on an eyeball replica and an artificial hand. Such sensor was applied in human sweat and was able to distinguish sweat glucose increase before and after a meal. Based on a similar principle, a flexible organic FET-based lactate sensor was demonstrated by detecting the produced H_2O_2 .^[144] This flexible transistor for electrochemical lactate sensing was realized by modifying LOx and the ferrocene mediator in a gel-format ionic liquid. As lactic acid was oxidized to pyruvate, lactate oxidase was reduced and regenerated by the mediator, which carried electrons to the gate electrode. This led to a decrease in the potential across the gate/electrolyte interface and a concomitant increase of the potential at the channel/electrolyte interface.

In addition to enzymatic metabolic sensing, flexible FETs have been successfully used in the detection of a specific electroactive analyte such as adrenaline, dopamine, and ascorbic acid in biological samples.^[145] Nanoelectric FETs based on large-scale micropatterns of conductive reduced graphene oxide films showed the capabilities in dopamine sensing, likely due to the doping effect resulted from strongly π - π interaction between dopamine.^[146] The flexible FETs were used for real-time monitoring of the secretion of dopamine from living neuroendocrine PC12 cells. Organic electrochemical transistors (OECT), with both channel and gate obtained by PEDOT:PSS deposition, were demonstrated as flexible chemical sensors on a textile structure by assessing various redox-active molecules such as adrenaline and dopamine. Such textile OECT-based sensor took advantage of the electrocatalytic features of PEDOT to oxidize the electroactive compounds.

The flexible FETs could also potentially be used as ion-selective sensors. Ionescu and co-workers recently introduced a wearable and flexible FET platform to track biochemical information such as pH, Na^+ , and K^+ at the surface of the skin in real time (Figure 6c,d).^[147] This microsystem heterogeneously integrated a biocompatible microfluidic interface, to deliver a “lab-on-skin” sensing platform. Takei and co-workers proposed a flexible charge-coupled device based on an ion sensitive FET for sweat pH sensing (Figure 6e,f).^[148,149] A flexible temperature sensor was integrated to monitor the skin temperature and to compensate the temperature effect on the response of the ion-sensitive FET. Multiple accumulation cycles of electron charge transfer were used to achieve enhanced the sensitivity toward wearable pH sensing. With 100 cycles of accumulation, a sensitivity of around 240 mV pH^{-1} were achieved, which passed the sensitivity limit imposed by Nernst theory.

6. Affinity-Based Flexible Electrochemical Biosensors

Affinity-based biosensor techniques have been widely used in biomedical applications for analyzing a broad spectrum of analytes, including proteins, peptides, hormones, and neurotransmitters.^[150] In general, affinity biosensors are analytical devices composed of a biological recognition element such as

the antibody, receptor protein, biomimetic material, or DNAs/RNAs interfaced to a signal transducer. The selectivity, sensitivity, and stability of the affinity-based biosensor are primarily determined by the bioaffinity elements. Enzyme-linked immunosorbent assays (ELISA) are the most commonly used affinity-based sensing approach in both research and clinical settings. However, such assays are time-consuming and require bulky analytical instruments (e.g., plate readers). Owing to its high sensitivity, selectivity, portability, and low cost, affinity-based electrochemical sensors hold great promise toward wearable and point of care applications.

Natural bioreceptors such as antibodies, lectins, and peptides have high selectivity to the target antigen due to the shape-selective recognition. Electrochemical sensors based on such recognition mechanism have been broadly demonstrated in biosensing over the past years. In recent years, research progress has been made in combination of antibody/peptide-based sensing and flexible electronics toward wearable and implantable sensing. Mannoor et al. reported a flexible and wireless graphene-based biosensor for bacteria detection on tooth enamel (Figure 7a).^[151] The fully integrated biosensing platform was fabricated by graphene printing onto water-soluble silk substrate, followed by functionalization of antimicrobial peptides (AMPs) as biorecognition moieties. The selective binding of the bacteria with the AMPs alters the electrical resistance that allows rapid bacterial quantification (Figure 7b). Wireless RF powering and readout was facilitated through an integrated resonant coil. Prasad and co-workers reported wearable and flexible immunosensors for the detection of cortisol, alcohol, as well as cytokines in artificial sweat samples. As an example, an alcohol consumption monitor was proposed based on the detection and quantification of ethyl glucuronide (EtG), a metabolite of ethanol.^[152] Monoclonal antibodies for EtG were immobilized on the Au or ZnO sensor electrodes using thiol-based chemistry. The binding of EtG with the antibodies on the electrode inhibited the charge transfer of a bound redox mediator and was monitored using electrochemical impedance spectroscopy and SWV.^[153] Kokkinos et al. developed an integrated foldable electrochemical device based on paper for duplex protein biosensing by using Pb- and Cd-based quantum dots (QDs) as labels.^[154] The bismuth modified graphite electrode as the working electrode was sensitive to the released metallic ions from QDs via duplex ASV determination, enabling the simultaneous quantification of bovine casein and bovine immunoglobulin G.

Aptamers are nucleic acids (DNA or RNA) that can bind to the target molecules with high selectivity and affinity similar to antibodies. Moreover, unlike natural antibodies, aptamers can be engineered and generated against a broad range of organic and inorganic small molecules ranging from hormones, proteins to metal ions. Thus aptamer-based flexible sensors have attracted tremendous attention toward wearable and implantable sensing.^[155] An approach for the label-free detection of inflammatory cytokines (e.g., $\text{TNF-}\alpha$) using an aptamer-functionalized, graphene-based field-effect transistor (GFET) nanosensor on a flexible, SiO_2 -coated substrate of the polymer polyethylene naphthalate was reported. This flexible nanosensor was able to detect $\text{TNF-}\alpha$ within 5 min with a limit of detection of 26 pmol .^[156] A highly sensitive FET biosensor array for dopamine sensing was developed through immobilization of

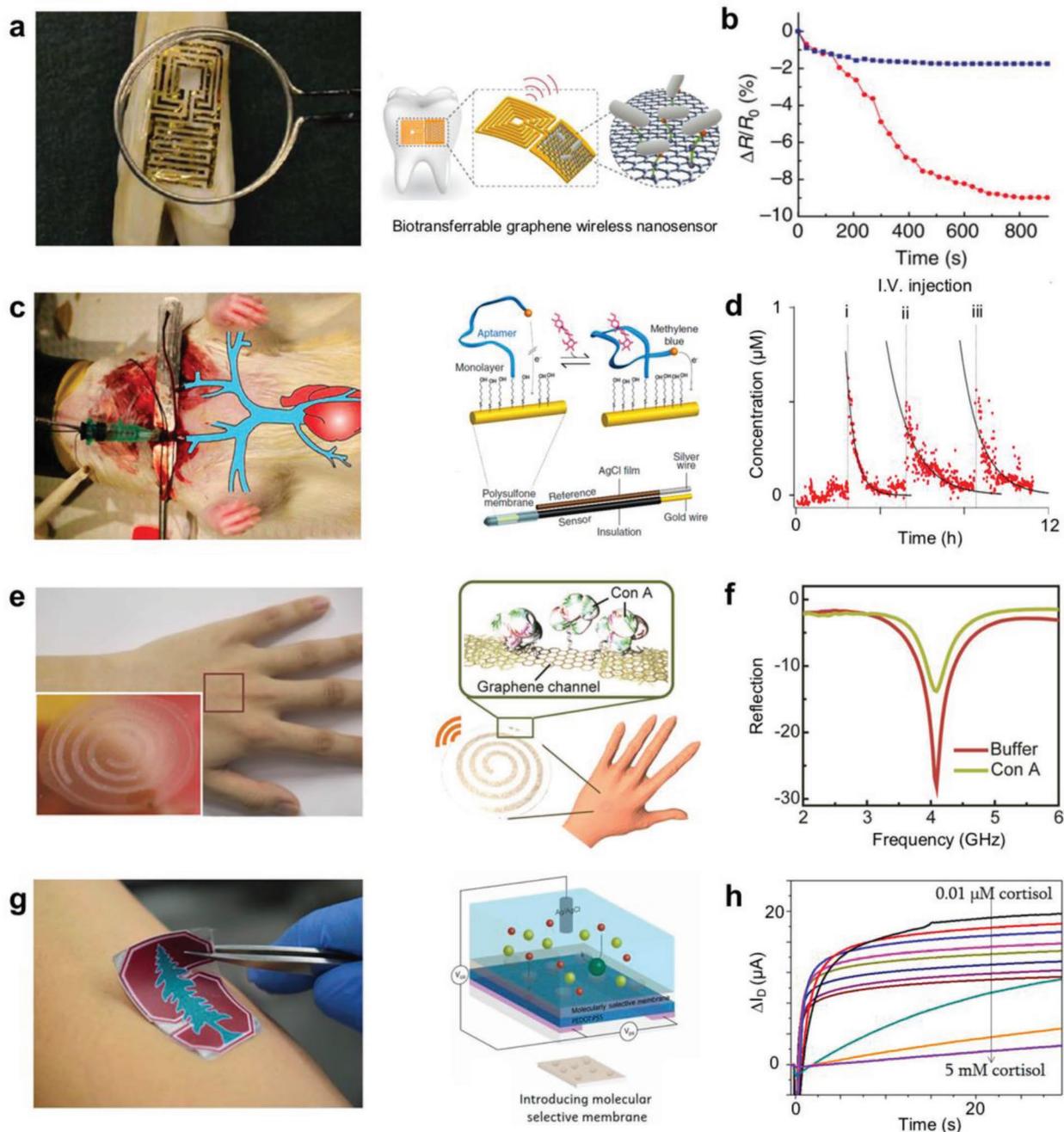


Figure 7. Affinity-based flexible electrochemical biosensors. a) A graphene-based antimicrobial peptides functionalized wireless sensor bacteria detection on tooth enamel. b) Relative resistance change of the graphene sensor upon exposure to *Helicobacter pylori* cells containing saliva (red) and blank saliva (black). a,b) Reproduced with permission.^[151] Copyright 2012, Springer Nature. c) An implantable aptamer-based sensor for real-time continuous drug monitoring. d) Blood levels of the drug tobramycin monitored using an implanted aptamer-based sensor in a wake, freely moving rat. c,d) Reproduced with permission.^[158] Copyright 2017, National Academy of Sciences, USA. e) A wireless FET sensor with Concanavalin A modified graphene channel for protein sensing. f) The measured reflection of the wireless FET sensor upon exposure to Con A and buffer. e,f) Reproduced with permission.^[163] Copyright 2015, Wiley-VCH. g) A flexible sensor patch based on molecularly imprinted polymers and an organic transistor for sweat cortisol sensing. h) The output current of the wearable cortisol sensor in artificial sweat with increased concentrations of cortisol. g,h) Reproduced with permission.^[166] Copyright 2018, American Association for the Advancement of Science. Reprinted/adapted from ref. [166]. © The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. Distributed under a Creative Commons Attribution NonCommercial License 4.0 (CC BY-NC) <http://creativecommons.org/licenses/by-nc/4.0/>.

specific aptamers to the channel surface.^[157] Thiol terminated tethered DNA aptamer, which selectively recognizes dopamine, was immobilized on ultrathin In_2O_3 surfaces via linkers to

complete the biosensor fabrication. The linear working range of the aptamer- In_2O_3 biosensor was determined to be 10^{-11} to 10^{-7} mol. Plaxco and co-workers recently reported the use of

aptamer functionalized flexible wires for continuous drug monitoring in vivo with clinically relevant sensitivity and high temporal resolution (<3 s) (Figure 7c,d).^[158] A covalently attached redox reporter, methylene blue (MB), was used to achieve the electrochemical signal transduction. SWV was used to continuously monitor the changes in electron transfer caused by the binding-induced folding of MB-modified aptamers. A label free laser-scribed graphene-based electrochemical thrombin biosensor was developed using amino-functionalized antithrombin aptamers.^[159] The thrombin/aptamer binding blocked the electron transfer from the redox marker, and was monitored through DPV.

Artificial receptors, such as molecularly imprinted polymers (MIPs), has also attracted attentions for the advantages of low cost, simple analysis process, good reproducibility, and high selectivity compare to their natural counterparts. MIPs are synthetic polymers containing tailor-made recognition sites. As an example, a urea-PEDOT/CNT/Au-based flexible wearable biosensor by electropolymerization in the solution of urea and EDOT forms MIP membranes.^[160] After removal thorough elution, the recombination of urea molecules with recognition sites led to the electron transfer resistance of potassium ferricyanide probe, realizing the selective urea determination.

Recent developments in affinity-based electrochemical FETs enable sensitive and label-free detection of biomarkers in a rapid and continuous manner.^[161] An affinity-based FET biosensor array for label-free detection of protein HIgG was demonstrated which relied on inkjet-printed source, drain, and gate electrodes using silver nanoparticles and antibody modified organic semiconductor channels.^[162] Figure 7e demonstrates a highly transparent and stretchable lectin (Concanavalin A) modified FET based on the graphene–Ag nanowire hybrid film for sugar and protein sensing.^[163] In this FET structure, the graphene layer was continuously and monolithically connected through all conductive components of the channels and electrodes, which significantly reduced the contact resistance and enhance FET mobility. The FET arrays were operated as real-time, wireless sensors for detection of carbohydrate binding proteins via glycosylation of the graphene channel. Utilizing the 2D materials (e.g., graphene and MoS₂) modified FETs, flexible sensors were reported toward selective detection of key biomarkers and cells for breast cancer and prostate-specific antigen (PSA) (Figure 7e).^[164,165] Through the combination of the MIPs and the electrochemical organic transistor technologies, a flexible skin-interfaced biosensor was reported recently for sensitive and selective monitoring of stress hormone cortisol in human sweat (Figure 7g).^[166] This MIP-based wearable cortisol sensor achieved a log-linear response in the range of 0.01–10.0 μmol (Figure 7h). The MIP-based FET sensor and a laser-patterned microcapillary channel array are integrated in a single wearable platform, providing accurate sweat sampling and precise sample delivery, as well as sweat cortisol analysis.

Affinity-based flexible sensors are emerging as a promising electrochemical platform for biosensing body fluidic applications due to the highly selective, low detection limit, miniaturable design, and the portability. However, the complex pretreatments of the affinity-based electrochemical biosensor led to low efficiency and high cost, which limit their use in the practical wearable application. Moreover, the bonding of

the analyte and bioreceptor is usually difficult to be regenerated, which means that the affinity-based biosensors are mostly only suitable for one-time use. Future research should focus on materials and technology innovations for reducing the assay steps and for sensor regeneration.

7. Flexible Biofuel Cells for Energy Harvesting and Self-Powered Sensing

With the rapid development of flexible and wearable bioelectronics which could provide rich information for real-time continuous health monitoring and early disease diagnosis, sustainable energy harvesting and storage from human body have become one of the most important research focuses in flexible electronics fields.^[167] Various kinds of flexible energy devices that could harvest energy from body heat, movement, solar energy, and body fluids have been developed to supply wearable electronics. Among these, biofuel cells, which can convert the chemical energy in the biological fluids into electrical energy by utilizing the redox enzymes as the bioelectrocatalysts, have attracted considerable attentions for wearable applications owing to its biocompatibility, sustainable green energy source, and high power intensity.^[168] Considering that our body fluids (e.g., blood, sweat, saliva, tear, as well as urine) contain rich levels of chemical fuels such as glucose, lactate, fructose and ascorbate, the power of the biofuel cell can be generated from both inside or outside based on various substrates. Moreover, flexible biofuel cells can operate as membraneless cells and can be directly applied on the skin owing to the high selectivity and biocompatibility of the enzyme itself.

7.1. Energy Harvesting Strategy Based on Flexible Biofuel Cell

Flexible biofuel cells have recently been developed toward continuous energy harvesting from the body fluids such as blood, sweat, saliva and tears.^[169,170] Glucose is one of the most abundant substrate for developing biofuel cells toward powering future implantable and wearable bioelectronics.^[171] Implantable biofuel cells based on enzyme modified flexible fibers have been widely reported for harvesting energy from living animals such as rats, snails, clams, lobsters and insect.^[172–176] For instance, a glucose/O₂ biofuel cell with a GOx modified bioanode and Pt nanoparticles modified cathode was implanted in a living rat.^[177] The implantable biofuel cell was able to generate a maximum output power of 95 $\mu\text{W cm}^{-2}$ and remained stable for around 24 h. For larger area as the wearable energy devices, a glucose/O₂ biofuel cell was constructed with a fully freestanding and flexible buckypaper (Figure 8a).^[178] Glucose dehydrogenase, as an oxygen-insensitive enzyme, could convert the glucose to gluconolactone through mediator electron transfer. In combination with the O₂ reducing bilirubin oxidase (BOD) cathode, the flexible biofuel cell reached 0.65 mW cm^{-2} under oxygen-saturated conditions (Figure 8b). Interestingly, glucose can also serve as the cathode substrate based on buckypaper through combining the glucose oxidase (GOx) with the horseradish peroxidase (HRP).^[179] On the cathode, HRP could convert the H₂O₂, the product of the GOx with oxygen, into

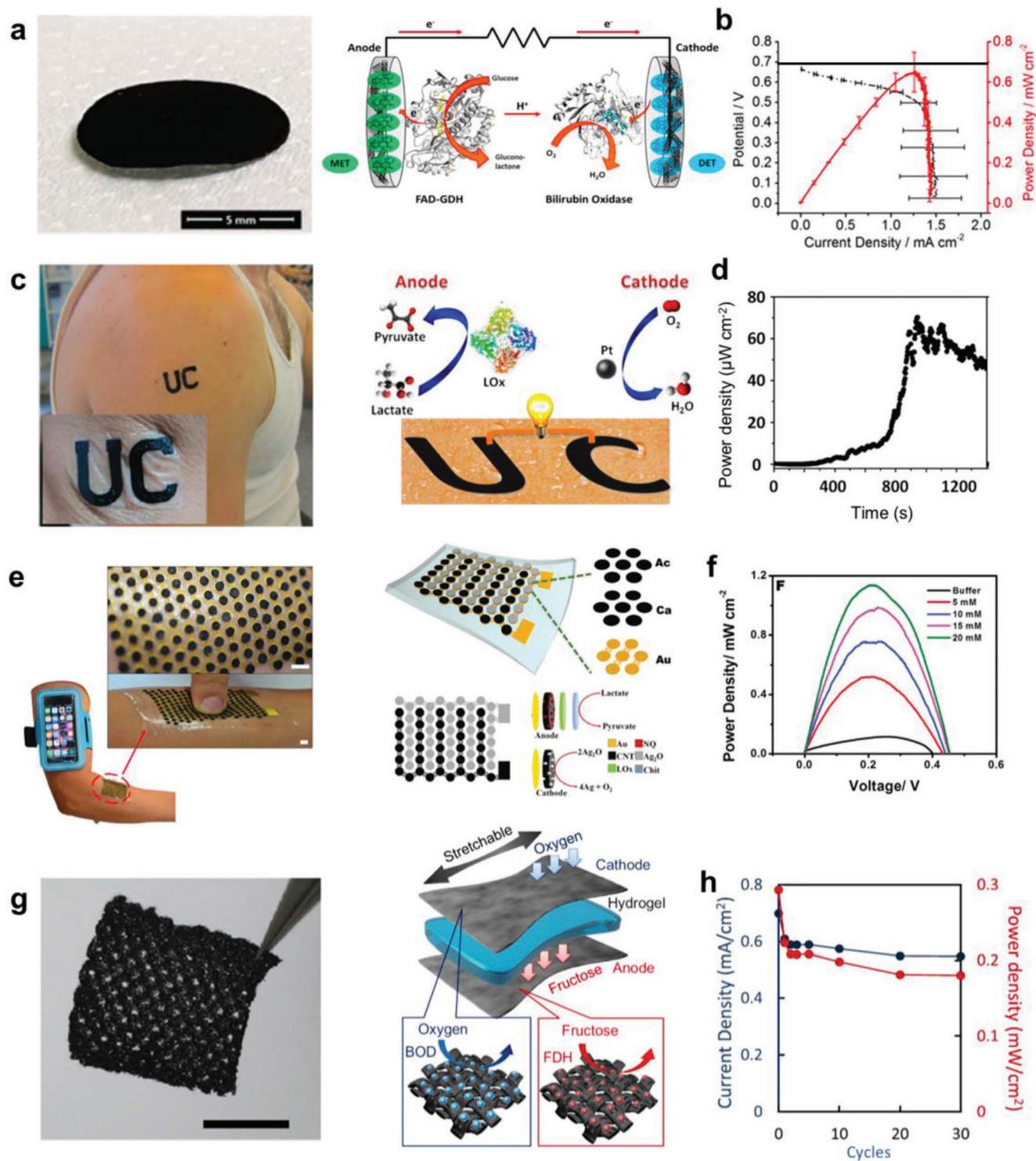


Figure 8. Flexible biofuel cell for energy harvesting from the human body. a) A buckypaper-based single compartment biofuel cell for harvesting energy from glucose. b) Biofuel cell polarization (black) and power curves (red) in McIlvaine buffer containing 170×10^{-3} M glucose. a,b) Reproduced with permission.^[178] Copyright 2017, American Chemical Society. c) A wearable tattoo-based biofuel cell utilizing sweat lactate as the substrate. d) Real-time power-density profiles of a tattoo-based lactate biofuel cell during a cycling exercise. c,d) Reproduced with permission.^[182] Copyright 2013, Wiley-VCH. e) A soft stretchable electronic skin-based biofuel cell with high power output extracted from sweat lactate. Scale bars, 5 mm. f) Power density curves of the stretchable biofuel cell recorded in lactate solutions. Scale bars, 5 mm. g) A CNT-modified stretchable biofuel cell based on fructose substrate. Scale bar, 5 mm. h) Current and power densities of the CNT biofuel cell on successive 50% stretching. g,h) Reproduced with permission.^[185] Copyright 2015, Elsevier B.V.

H₂O by accepting external electrons. The freestanding materials were outstanding for assembling the flexible and wearable biofuel cells with low cost and flexibility such as the origami and paper-based devices. A low-cost screen printing technology for large-scale fabrication of printable CNT-based biofuel cell was proposed, which was achieved by assembling the GOx as bioanode and Pt as cathode. The printable CNT-based polyurethane offered high stretchability up to 500%. The important novel aspect of the fabrications is the judicious preparation of a highly stretchable platform and its combination with judiciously designed device pattern like the supercapacitor.

Sweat lactate, excreted at high concentrations ($5\text{--}60 \times 10^{-3}$ M) as a metabolic product, is an ideal substrate for designing epidermal biofuel cells.^[180,181] Jia et al. developed a temporary tattoo-based biofuel cell comprising of a CNT/LOx modified bioanode and a platinum black-functionalized cathode (Figure 8c).^[182] The improved fuel cell efficiency, TTF/carbon nanotube composite was used as electron shuttle and a chitosan biocompatible layer was added on the anode to prevent current leakage to skin. The thin tattoo-based biofuel cell could withstand repeated mechanical deformations without compromising on the performance. The tattoo biofuel cells were applied on the skin of the human subjects to generate power real time (Figure 8d). The device was capable of repeated usage, as indicated of the power density generated at two sessions separated by a 2 h interval. The same group later applied this lactate-oxygen fuel cell system onto textile platform.^[183] A nonabsorbing textile was used to prevent fuel absorption; TTF-TCNQ complex was additionally used on anode to minimize oxygen competition in lactate oxidation by LOx. When four biofuel cells were connected in parallel in the headband, a blue LED could be powered with the energy harvested from a perspiring individual exercising on a stationary bike. The biofuel cells developed were mostly rigid electrodes attached to a flexible platform, and the discrepancy of mechanical compliance could strain the active energy harvesting layers and damage device performance. To improve the mechanical compliance of the overall device, a soft stretchable electronic skin-based biofuel cell with high power output was developed by Bhandodkar et al. (Figure 8e).^[184] The Au bridge-island pattern was designed to possess a close-packed structure between anodes and cathodes, while the interdigitated electrode pattern allows anodes and cathodes to be closely located for reducing internal resistance. The bridge-island structure has hexagonal close-packed pattern to maximum active area. Both cathode and anode islands comprised of compact 3D carbon nanotube-electrode material structure; the porous feature of such structure enables high loadings of active material. The high loading due to the 3D structure and the use of Ag₂O as cathode reactant contributed to the high power output achieved by this device (nearly 1.2 mW cm^{-2} at 0.2 V with an open circuit voltage of 0.5 V) (Figure 8f). When applied directly onto human skin, the fuel cell generates around 1 mW cm^{-2} during exercise.

Beyond glucose and lactate fuels, biofuel cells could potentially harvest energy from other substrates such as urea and fructose. A stretchable biofuel cell was demonstrated by laminating D-fructose dehydrogenase (FDH)-modified and BOD-modified conductive textiles as bioanode and biocathode (Figure 8g,h).^[185,186] A hydrogel film containing electrolyte and

biofuel in the middle of the electrodes. During the stretching of the textile, the base fibers (nylon/polyurethane cofiber) were not significantly expanded. As a result, the CNTs and enzymes were stable on the fibers and could maintain their performance even under continuous stretching test.

7.2. Self-Powered Flexible Electrochemical Biosensor Based on Biofuel Cell

Currently, a major challenge associated with miniaturization of the flexible bioelectronics is the sustainable power supply. The requirement for replacement or recharging of the batteries processes has greatly hindered the development of the flexible bioelectronics for wearable and particularly implantable use.^[187] Using biofuel cells as self-powered electrochemical biosensors, which merges the biosensing transducer with the sustainable power supply, represent an attractive solution for designing future self-powered bioelectronics.^[188–190] Highly stretchable textile-based BFCs as self-powered sensors were recently reported to extract electrical power from perspiration for probing the sensing events of sweat metabolites.^[191] The bioanode was modified by GOx or LOx enzyme for the biocatalytic oxidation of biofuels. A silver oxide/silver (Ag₂O/Ag) redox couple electrode was chosen as the consumed cathode. A linear relationship between the power output or the short circuit current (SCC) and the lactate concentrations up to 20×10^{-3} M was obtained. On body lactate sensing was achieved during cycling exercise through continuous monitoring of the short circuit current responses of the BFCs. Shleev's group made a micro-scale membraneless biofuel cell by utilizing the ascorbate and oxygen naturally present in tears as fuel and then integrated with glucose-sensing contact lenses for continuous health monitoring by diabetes patients.^[189] A self-powered flexible electrochemical biosensor was constructed based on a photochemical biofuel cell using a LOx modified bioanode and a photocathode covered by organic semiconductor polyterthiophene.^[192] This flexible BFC-based biosensor was able not only to detect the illuminance by the open-circuit voltage, but also to monitor the perspiration lactate by the SCC accurately and simultaneously. A positive linear relationship between OCV of the biofuel cell and the logarithm of the ambient illuminance was observed.^[193] Considering that enzyme activities can be inhibited by heavy metals, Wang et al. fabricated a paper-supported glucose/O₂ biofuel cell-based self-powered miRNA-21 sensor with colorimetric readout. Therefore, miRNA-21 worked as a key to unlock the Cu²⁺@MSN-DNA cap via triggering the release of Cu²⁺ which will inhibit the catalytic activity of the GOx. The power output of BFC decreased with the increase of the miRNA-21. Regarding the biofuel cell as the external power source to supply and cooperate with biosensors would be a novel strategy for assembling the self-powered electrochemical biosensor.^[194]

Despite exciting progress on biofuel cell and self-powered sensor development, there are several limitations to be addressed. First, since most biofuel cells were based on enzyme reaction, long-term stability of the enzymes and redox mediator (if present) was necessary for proper function of the biofuel cell. Changes in environment conditions such as temperature, humidity could affect enzyme structure and catalytic

activities. Stabilization of the enzymes through engineering the enzyme modification as well as applying enzyme stabilizers should be carefully considered. Second, the power output from noninvasive biofuel cells is still insufficient to power many wearable electronic devices. Expanding the electroactive area by introducing 3D materials and increasing the electrocatalysis activities of enzymes through protein engineering are the promising directions for improving the performance of biofuel cells. Constant power output requires sustained high level of fuel flow, which is difficult to achieve since the fuel flow is limited by physiology process. Sensing device with lower power consumption may be useful to alleviate such power shortage; using multisource energy harvest may be also promising. The third issue is the mechanical stress present due to physiological contours and resulted strain and damage on harvesting structure. To resolve damage-induced failure, self-healing all-printed electrochemical device could be designed. Better material selection and structure design will also be efficient in overcoming this challenge. Such flexible biofuel cells could also be combined with other energy harvesting strategies (e.g., solar cell, thermoelectric generator, piezoelectric and triboelectric nanogenerator) and energy storage devices (e.g., flexible supercapacitors or Li-ion batteries) to maximize their benefits in powering future bioelectronics.

8. Flexible Hybrid Bioelectronics with Electrochemical Technique

Electrochemical bioelectronics, as a rapid, sensitive and selective technique, could integrate with other techniques to form hybrid systems with new functionalities. For example, Bhandekar et al. recently demonstrated a battery-free flexible and wearable system that combined the self-powered BFC sensing with colorimetric and volumetric analysis (Figure 9a).^[96] The resulting device merged advantages of electronic and microfluidic functionality in a single platform with the capabilities of simultaneous detection of sweat rate, pH, lactate, glucose, and chloride. The collective physiological relevance of these parameters allowed for comprehensive tracking of an individual's health status. For the BFC-based glucose and lactate sensors, the enzymes LOx and GOx were immobilized on a conductive, high-surface-area carbon nanotube paper with the redox mediator tetrathiafulvalene for shuttling electrons. The cathodes were functionalized with platinum black, and then coated with a Nafion membrane. The current output of the BFCs was transformed into a voltage-based signal that can be transmitted wirelessly to a cellphone via the near field communication technology. Colorimetric chloride and pH tests relied on silver chloranilate (a chemical that complexes with chloride ions to generate a species with a distinct purple color), and a pH-sensitive dye, respectively.

Recently, the rapid development of wearable electronics has resulted in devices that can monitor physiological information such as body motion, respiration, heart rate, electrocardiograms, skin temperature, and blood pressure. In order to provide a complete picture of the health state of an individual, it is very attractive to measure both physiological changes and the molecular information using hybrid wearable systems. Skin

temperature, as an important vital sign, has been monitored integrated with electrochemical bioelectronics. Such measured skin temperature information could also be used in real time to calibrate the readings of the electrochemical sensors to achieve high in situ detection accuracy. In another example, Imani et al. proposed a wearable hybrid device that could simultaneously measure both chemical and electrophysiological parameters in a single epidermal patch (Figure 9b).^[195] The hybrid wearable device comprised a screen-printed amperometric lactate biosensor and two electrocardiogram electrodes for concurrent real-time measurements of lactate and electrocardiogram. A hybrid and wireless sensing system was recently reported based on soft contact lenses for simultaneous intraocular pressure sensing and electrochemical tear glucose sensing.^[196] Graphene/Ag nanowire nanostructure was used to pattern the FET electrodes and the antenna, while the glucose sensing was facilitated by using GOx modified graphene as the channel.

Flexible electrochemical bioelectronics holds great promise for future wearable and point of care disease prevention and control, and healthcare monitoring in nonlaboratory settings without well-trained technicians. The closed loop of sensing and treatment could significantly improve the quality of healthcare management. Kim and co-workers integrated a flexible hybrid patch that contained a multiplexed sensing component for humidity, glucose, pH, and temperature sensing, and a therapeutic component with insulin-loaded microneedles (Figure 9c).^[101] High glucose concentration recordings triggered the embedded heaters in the therapeutic component. As a result, bioresorbable microneedles could be dissolved under elevated temperature and release Metformin as a feedback transdermal drug delivery. The use of intrinsically soft materials enhanced the conformal integration of devices with the human skin and thus improved the effectiveness of biochemical sensors and drug delivery.

9. Fabrication of the Flexible Electrochemical Bioelectronics

Flexible electrochemical bioelectronic devices could be readily prepared using a number of fabrication methods. Parameters to consider when choosing fabrication technologies include the electrode materials, substrate, device dimension, cost, uniformity, as well as electrochemical performance. Although standard semiconductor manufacturing techniques such as photolithography and thin film evaporation have been broadly used in preparing bioelectronic devices, moving toward practical biomedical applications, there is a strong need for high-throughput and low-cost fabrication of flexible devices with high electrochemical performance. In this regard, a number of printing approaches have attracted tremendous attention including screening printing, inkjet printing, roll-to-roll printing, 3D printing as well as laser printing.^[197,198]

Screen printing, a printing technique using a mesh to transfer ink onto a substrate, is a most commonly used technique for the fabrication of electrochemical sensors. Screen printing has distinct advantages: it is suitable for a wide spectrum of substrates including textiles, paper, plastics, as well as wood; it is very cost effective, particularly for bulk orders; the

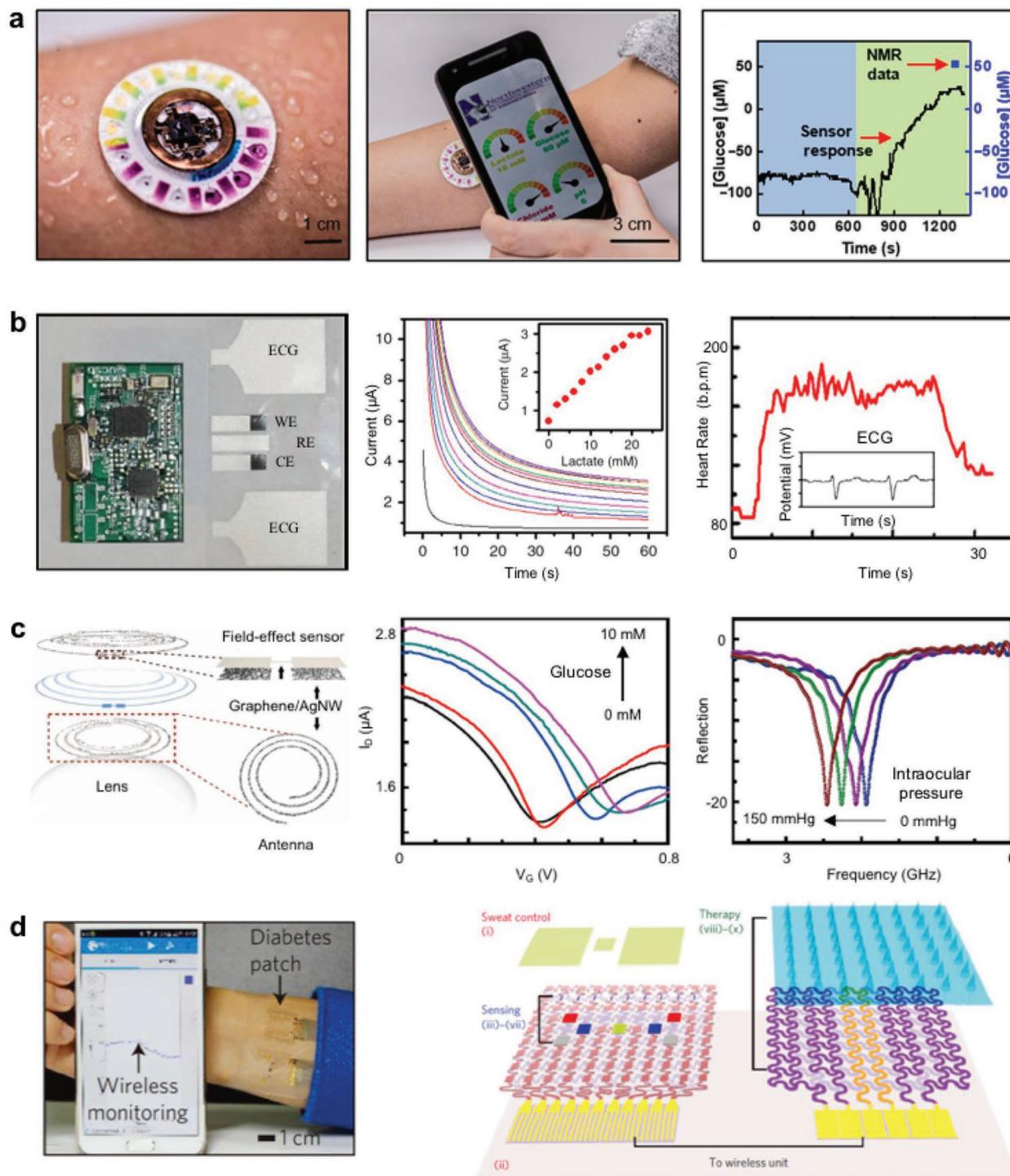


Figure 9. Flexible hybrid bioelectronics. a) A hybrid battery-free sweat sensing system that integrates the self-powered BFC-based sensing with colorimetric and volumetric analysis. Reproduced with permission.^[96] Copyright 2019, American Association for the Advancement of Science. Reprinted/adapted from ref. [96]. © The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. Distributed under a Creative Commons Attribution NonCommercial License 4.0 (CC BY-NC) <http://creativecommons.org/licenses/by-nc/4.0/>. b) A chemical–electrophysiological multimodal wearable sensor patch that can simultaneously monitor sweat lactate and electrocardiogram. Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>).^[195] Copyright 2016, Springer Nature. c) A wearable contact lens sensor for simultaneous intraocular pressure sensing and electrochemical tear glucose sensing. Reproduced under the terms of the CC-BY Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>).^[196] Copyright 2017, The Authors, published by Springer Nature. d) A wearable platform for sweat-based diabetes monitoring and feedback therapy. Reproduced with permission.^[93] Copyright 2016, Springer Nature.

screen-printed electrodes usually have very high-quality electrochemical output. Screen-printed electrodes have been successfully used in a number of medical diagnosis applications (glucose strips for diabetes management in particular). Using screen printing technology, Wang and co-workers successfully prepared a wide range of the flexible and wearable tattoo- and textile-based sensors for analyzing analytes of interest in blood, sweat, interstitial fluid, as well as saliva (**Figure 10a**).^[94,180,199] Through engineering the ink composition and printing

substrate, all printed stretchable and healable flexible electrochemical sensors and biofuel cells were demonstrated.

Inkjet printing, a droplet-based printing technology, has attracted more and more attention recently years.^[200] Unlike screen printing, inkjet printing has low setup cost without the need of the template or stencil. Thus it could offer rapid printing with well-controlled patterns as well as simultaneous multi-ink printing, which are ideal for prototyping or the production of small-scale flexible electronic devices.^[201] Moreover, as the

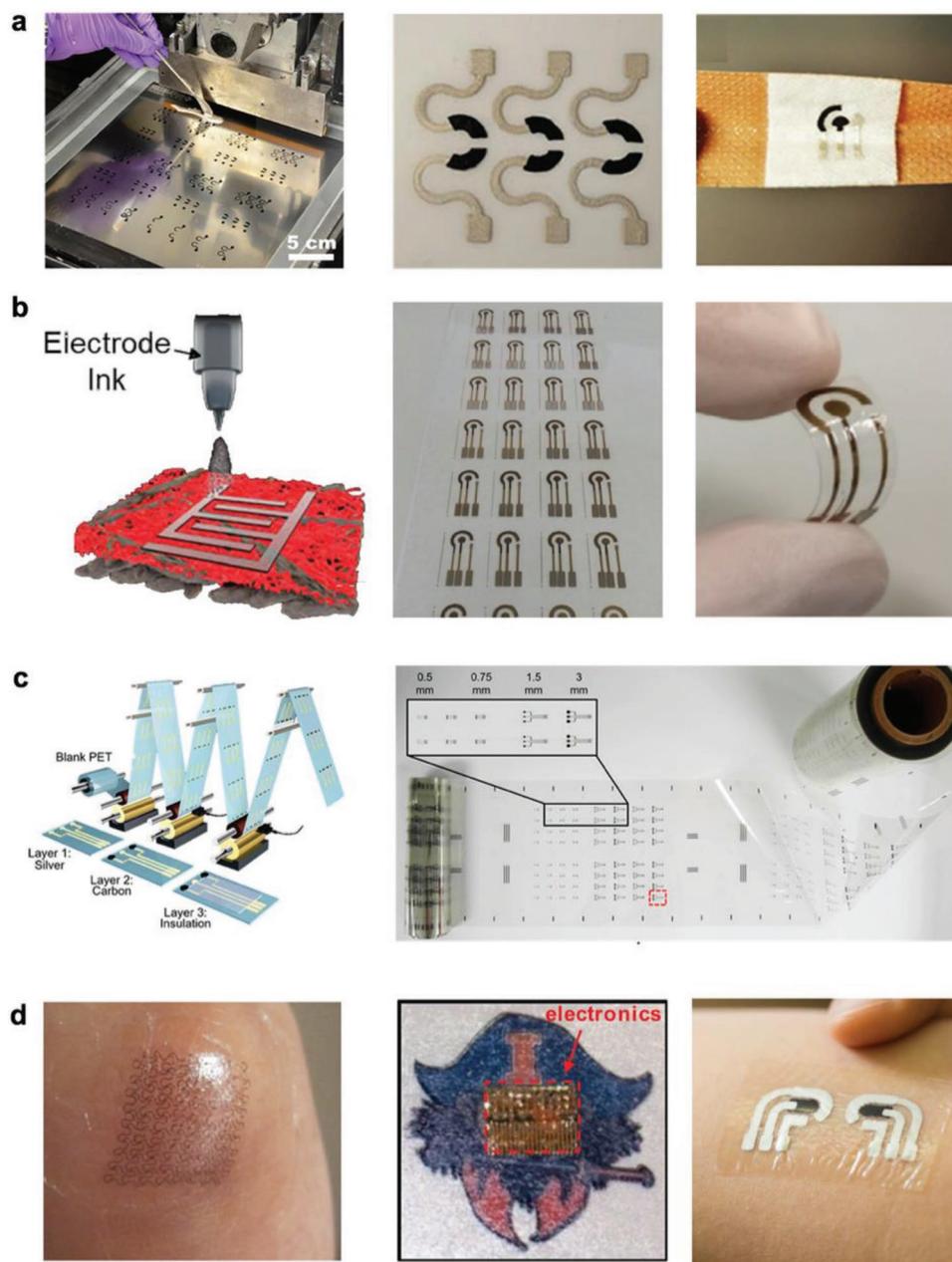


Figure 10. Fabrication of the flexible electrochemical bioelectronic devices. a–c) Large-scale and low-cost fabrication of the flexible electrochemical sensors. a) Screen printing. Left: Reproduced with permission.^[180] Copyright 2016, American Chemical Society. Middle and right: Reproduced with permission.^[199] Copyright 2018, Wiley-VCH. b) Inkjet printing. Left: Reproduced with permission.^[201] Copyright 2016, The Royal Society of Chemistry. Middle and right: Reproduced with permission.^[204] Copyright 2018, Elsevier. c) Roll-to-roll printing. Reproduced with permission.^[206] Copyright 2018, American Chemical Society. d) Transfer printing technology to prepare flexible epidermal tattoo based electrochemical devices. Left and middle: Reproduced with permission.^[2] Copyright 2011, AAAS. Right: Reproduced with permission.^[94] Copyright 2015, American Chemical Society.

development of the high-end printers (such as Dimatix Materials Printer), the ink drop size, printing spacing, printing speed, and ink drying process can be adjusted according to the specific application.^[202,203] Owing to these advantages, a number of inkjet-printed flexible analytical devices have been reported toward wearable and point-of-care analysis (Figure 10b).^[204] It should be noted that inkjet printing has a limited range of printable substrates and could still suffer in terms of scalability and throughput.

For extremely large-scale and high-throughput fabrication of the flexible electrochemical bioelectronics, roll-to-roll printing would be more applicable which involves a process of creating electronic devices on a roll of flexible substrate.^[205] Figure 10c illustrates the use of roll-to-roll to print high-performance flexible sensing electrodes on 150 m flexible substrate rolls. These sensors demonstrated excellent electrochemical performance toward wearable sweat sensing.^[206]

In addition to the above-discussed ink-based printing technologies, transfer printing represents an emerging technology that enables the heterogeneous integration of various electronic materials into the desired flexible and/or stretchable substrate. Considering that the fabrication and patterning of the inorganic semiconducting or metallic materials often require extreme processing conditions (e.g., high temperature), transfer printing allows the electronic system to be fabricated on wafer/donor substrate followed by the assembly onto the flexible/stretchable receiving substrates (e.g., silicone rubbers), bypassing the compatibility issues between the polymeric substrate with conventional fabrication technologies. Transfer printing has been widely used in the fabrication of the new classes of soft bioelectronics.^[207] For example, the flexible and/or stretchable epidermal tattoo-based wearable platforms for continuous physiological health monitoring and electrochemical sweat analysis were demonstrated recently by Rogers group and Wang group, respectively, which involved the transfer printing of the lithography or screen printed electrodes onto the soft polymeric substrate (Figure 10d).^[2]

10. Conclusion and Outlook

Herein, we have summarized and highlighted most prominent electrochemical approaches in flexible bioelectronics toward personalized health monitoring which include potentiometry, amperometry, controlled-potential voltammetry, field-effect transistors, as well as biofuel cells. Electroanalytical methods, serving as a critical and unique technique in the emerging wearable and implantable bioelectronics, have received tremendous attention in the past decade. In order to achieve selective and sensitive biosensing in raw biofluids, proper electrochemical approaches should be carefully chosen and engineered along with the materials and device development. A number of innovative flexible electrochemical sensing devices have been reported recently toward analyzing the biomarkers of interest (ranging from metabolites, electrolytes, heavy metals, drugs, to neurotransmitters, proteins, and hormones) in a variety of body fluids including blood, ISF, saliva, CSF, tear, and sweat. Wearable biofuel cells have been demonstrated as a promising approach to power future bioelectronics devices in a suitable fashion. Such progress has demonstrated the promising future of such flexible

electrochemical bioelectronics in practical real-world applications in both fundamental and clinical aspects.

Despite the significant progress in the field of flexible electrochemical bioelectronics, many key challenges need to be addressed. First, most existing wearable and implantable flexible biosensors are focusing on detecting a very limited number of biomarkers (common metabolites and electrolytes) in body fluids. In fact, the body fluids contain abundant biomarkers (e.g., proteins, hormones, and peptides) in trace amounts which have close relationships with various health conditions. The detection of these biomarkers commonly relies on affinity-based recognition which is hard to regenerate in vivo with current technologies. Second, current flexible bioelectronics devices face major technological gaps related to their electrochemical selectivity, sensitivity, stability, repeatability as well as mechanical reliability and system robustness when used continuously in body fluids. Factors such as the fluid pH, volume, and flow rate could potentially affect the accuracy of in situ monitoring and proper calibration mechanisms need to be introduced to achieve better accuracy. Furthermore, although nanomaterials play an important role in flexible electrochemical bioelectronics toward improving the sensing performance, their potential toxicity and biocompatibility need to be carefully evaluated. Finally, a major bottleneck lies in understanding the physiological relevance of the target analytes to determine their utility in practical health monitoring and management applications. Addressing these challenges through materials, device and system innovations as well as large scale in situ studies is critical for the future success of the electrochemical bioelectronics and requires materials and technological innovations.

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Conflict of Interest

The authors declare no conflict of interest.

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