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Chapter

Wearable Skin-Worn Enzyme-Based Electrochemical Devices: Biosensing, Energy Harvesting, and Self-Powered Sensing

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Abstract

Integrating enzymes with wearable electrochemical systems delivers extraordinary functional devices, including biosensors and biofuel cells (BFCs). Strategies employing enzyme-based bioelectronics represent a unique foundation of wearables because of specific enzyme recognition and catalytic activities. Therefore, such electrochemical biodevices on various platforms, e.g., tattoos, textiles, and wearable accessories, are interesting. However, these devices need effective power sources, requiring combining effective energy sources, such as BFCs, onto compact and conformal platforms. Advantageously, bioenergy-harvesting BFCs can also act as self-powered sensors, simplifying wearable systems. Challenges pertaining to energy requirements and the integration of biocatalysts with electrodes should be considered. In this chapter, we detail updated advancement in skin-worn devices, including biosensors, BFCs, and self-powered sensors, along with engineering designs and on-skin iontophoretic strategies to extract biofluids. Crucial parameters including mechanical/material aspects (e.g., stretchability), electrochemistry, enzyme-related views (e.g., electron shuttles, immobilization, and behaviors), and oxygen dependency will be discussed, along with outlooks. Understanding such challenges and opportunities is important to revolutionize wearable devices for diverse applications.

Keywords: wearable technology, electrochemical devices, enzyme-based bioelectronics, biosensors, biofuel cells, self-powered biosensors, sweat, iontophoresis, personalized healthcare

1. Introduction

Since 1962 when the first Clark's biosensor was introduced [1], enzymatic electrochemical devices have attracted increasing attention, recently being regarded as a powerful tool for the development of emerging wearable bioelectronics [2]. Integrating enzymes with electrochemical transduction units is one of the most popular and well-built bioelectronic systems due to outstanding selectivity and natural behaviors of enzymes [2–4]. Employing enzymes, as a catalytic system, in order to substitute nonselective metal catalysts, is interesting. Because of inherent behaviors of enzymes, enzyme-based bioelectronics offers favorable operations

under mild physiological conditions of pH and temperature, unlike nonenzymatic approaches [5, 6]. In addition, enzymes will usually catalyze only one particular reaction. Therefore, such enzyme specificity enables bioelectronics to operate selectively even in complex solutions, including biofluids. Recently, there is an increasing interest in transforming traditional enzymatic bioelectronics into modern wearable platforms. Wearable enzyme electronics expands appealing spectra of a variety of applicable fields, ranging from personalized healthcare, fitness, to the environment. These applications comprise of noninvasive diagnosis of biomarkers in biofluids, such as sweat, and the monitoring of the surrounding of the wearer. Besides, electron collectors can be functionalized with enzymes to develop BFCs for energy and self-powered applications. These biodevices employ enzymes to obtain electrocatalytic oxidations of biofuels, such as glucose and lactate. This aims to achieve next-generation energy autonomy for the whole wearable system. In addition to energy-harvesting purposes, BFCs can also act as self-powered electrochemical sensors. Three main applications of enzyme-based
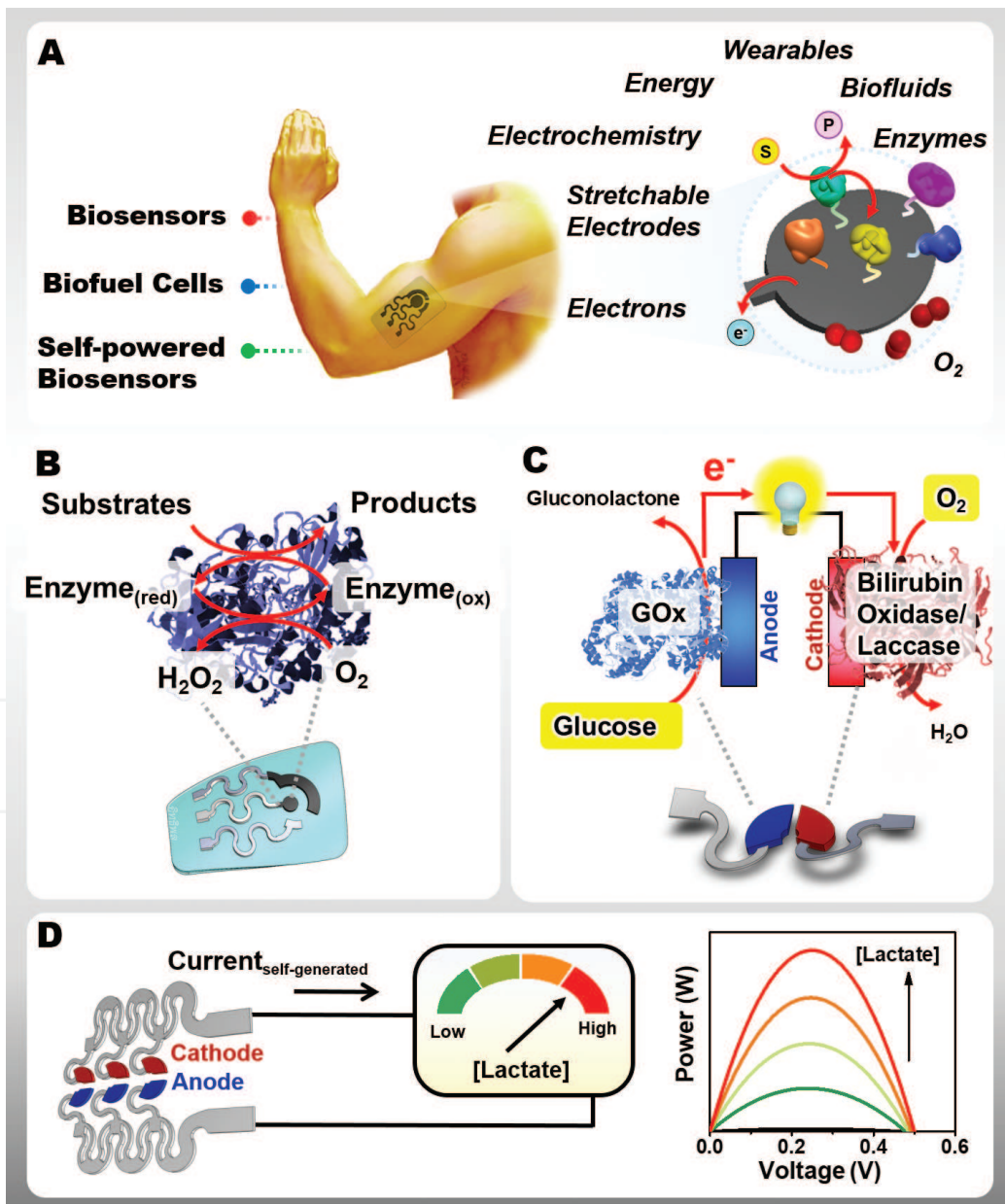

Figure 1.
(A) Skin-worn enzyme-based electrochemical devices. The soft electrode platform is functionalized with enzymes, allowing various applications, including (B) biosensors, (C) energy-harvesting biofuel cells, and (D) self-powered biosensors.

electrodes, including biosensors, biofuel cells (BFCs), and self-powered sensors, along with their relevant aspects, will be discussed (**Figure 1**). An enzymatic biosensor employs an enzyme, immobilized on an electrochemical transducer, to recognize and react with the target, generating a readable electrical signal (**Figure 1B**). A BFC energy harvester can convert chemical energy into electricity and power wearable devices (**Figure 1C**) [7]. A BFC can also be designed to act as a self-powered sensor by displaying power signals proportional to the target concentration (**Figure 1D**) [8, 9].

Skin-worn enzyme-based electrochemical devices are among the most significant wearables because the skin offers the largest organ interface and unique opportunities to be accessed noninvasively [10–13]. The large epidermal area also provides sweat, which contains a variety of biomarker-rich information, such as levels of glucose, lactate, hormone, urea, pH, and electrolytes. Advantageously, skin-worn electrochemical devices can be attached directly close to the location of sweat generation, enabling the fast access for monitoring or energy harvesting before the unwanted biodegradation. In addition to physical parameters obtained from existing skin-worn biodevices (such as temperature and heartbeat), chemical data is also crucial to step further to understand comprehensive insights of individual [14]. The history of sweat content analysis began many decades ago with the development of cystic fibrosis diagnosis [15]. Establishing new "lab-on-skin" electrochemical devices enables noninvasive detection of such biometrics, essential for health monitoring and early disease diagnosis. In addition, such wearable electrochemical tools are also helpful for drug testing and chemical threat screening, such as in sports [12] and in the surrounding environment [16]. Importantly, for emerging energy technologies, sweat also contains relevant biofuels, such as glucose and lactate; this is useful to BFCs as energy-harvesting and self-powered devices, which exemplify new exciting wearable autonomous bioelectronic systems.

Although researchers are battling to create new enzymatic bioelectronics, there is a continuing need for further development. Revolutionizing traditional electrodes toward wearable bioelectronics needs careful engineering to address several key challenges associated with electrochemistry, the integration of biocatalysts, mechanical stability, environment effects (e.g., O_2 fluctuations), and sweat extraction. Therefore, the bulk of this chapter will focus on examples of progress in skin-worn enzymatic electrochemical devices. Key working principles and opportunities of biosensors and BFCs will be described. In addition, perspectives emphasizing on main challenges will be discussed. The outlooks of emerging wearable electrochemical technologies will also be concluded.

2. Skin-worn enzyme-based electrochemical devices

2.1 Enzyme-based biosensors

Wearable enzymatic electrochemical biosensors utilize enzymes, which are functionalized in spatial contact with electrochemical transduction units. In principle, biosensors consist of electrodes and enzyme receptors, allowing the specific binding capabilities and catalytic activity to target analytes. Interfacing enzymes with electrodes will be discussed further in Section 3.3. It should be remarked that the key consideration to fabricate a successful biosensor for nonspecialist wearers is choosing highly specific biocatalysts. Enzymatic biosensors can also function continuously because enzymes are not consumed in reactions, offering an advantage for wearable sensors.

Enzymatic biosensors are based on numerous mechanisms. The popular mechanism relies on the conversion of the analyte as an enzymatic substrate into a product, enabling the detection by using electrochemical transducer. Another way is to monitor the analyte (e.g., a toxic compound) that acts as an enzyme inhibitor. In addition, the enzyme can be used as a labeling transducer for bioaffinity recognition. Besides, a reverse approach can be designed to detect the enzyme level. In this case, the enzyme acts as an analyte, while the substrate is immobilized on the electrode surface. When the enzyme reaches the electrode sensor, it will generate the signal, corresponding to the concentration level of the enzyme target.

In recent decades, enzymatic biosensors have been proven to be modern wearables to monitor numerous analytes, such as glucose, lactate, alcohol, and organophosphate nerve agents. Among several enzymes, oxidoreductase and hydrolase, such as glucose oxidase (GOx), lactate oxidase (LOx), alcohol oxidase (AOx), and organophosphorus hydrolase, are predominant for wearable biosensing applications. A temporary tattoo with the integration of transdermal enzymatic glucose biosensor has been introduced since glucose is a key biomarker for diabetes mellitus, which still affects hundreds of millions of patients globally (**Figure 2A**) [17]. The iontophoretic ISF extraction system was coupled with the amperometric
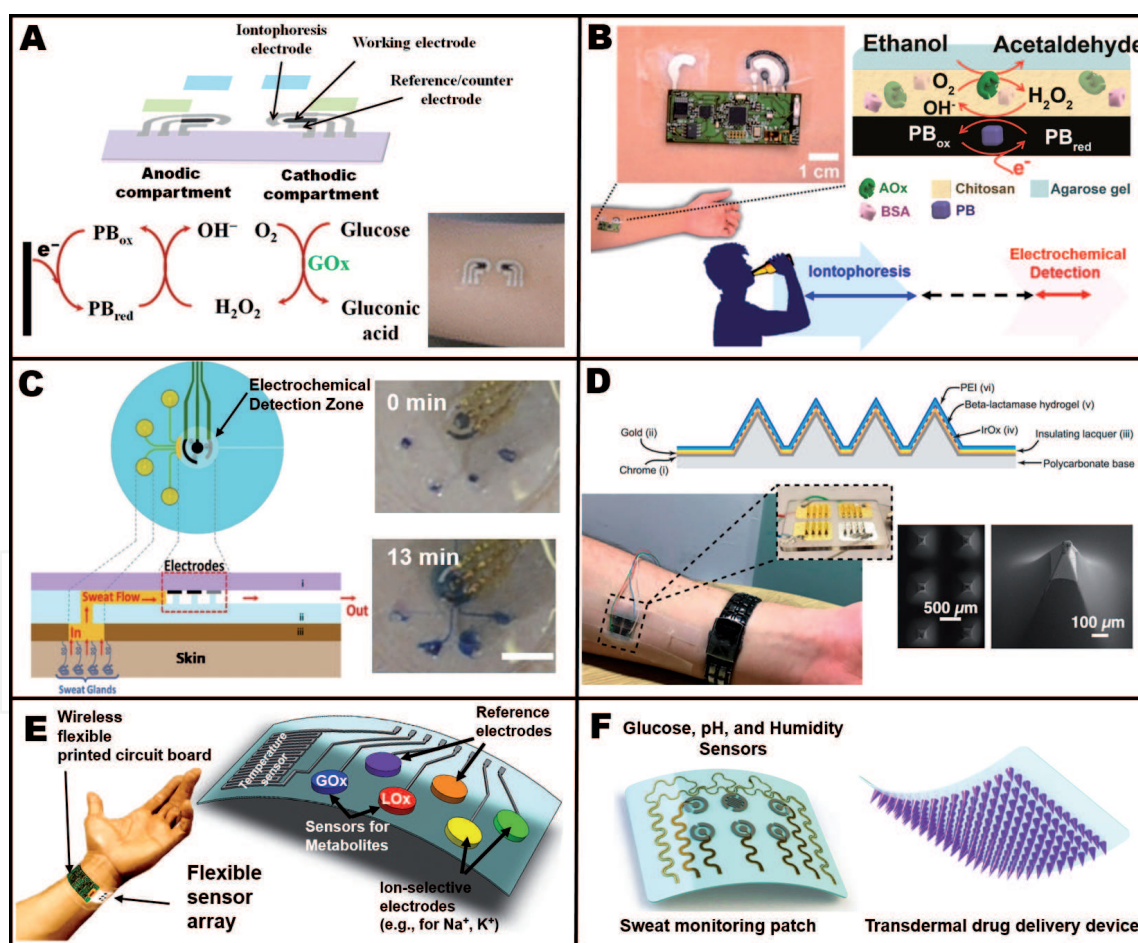

Figure 2.
Skin-worn enzyme-based electrochemical biosensors. (A) Transdermal tattoo-based glucose sensors, coupled with reversed iontophoresis [17]. Adapted with permission from ref [17]. Copyright 2015 American Chemical Society. (B) Tattoo-based alcohol biosensors, coupled with pilocarpine iontophoresis and wireless electronics [18]. Adapted with permission from ref [18]. Copyright 2016 American Chemical Society. (C) Biosensors integrated with a microfluidic patch for sweat collection and analysis [20]. Adapted with permission from ref [20]. Copyright 2017 American Chemical Society. (D) Microneedle-based β-lactam sensors [22]. Adapted with permission from ref [22]. Copyright 2019 American Chemical Society. (E) Integrated glucose/lactate enzymatic biosensors with electrolyte and temperature sensors. (F) Integrated sweat monitoring biosensing and transdermal drug delivery system.

detection to extract the sample containing glucose. The glucose biosensor, located near the negative iontophoretic electrode, relied on GOx immobilization on the Prussian blue (PB)-carbon electrode; this PB facilitates the electroreduction of H_2O_2 product, generated by the GOx reaction. The amperometric reduction of H_2O_2 could be detected at a potential of -0.1 V versus Ag/AgCl. The iontophoresis strategy will be discussed in Section 3.5. Additionally, the tattoo-based alcohol sensor was also invented (**Figure 2B**). The AOx-/PB-based sensor was designed to be close to the positive iontophoretic electrode to determine ethanol in sweat induced by transdermal delivery of the pilocarpine drug [18]. Moreover, recent efforts have been made to combine these two concepts, including glucose and alcohol sensors, on a single tattoo [19]. This holds a possibility for multianalyte sweat analysis.

Skin-worn microfluidic devices can enable the continuous flow of renewed sweat over operational periods. This addresses the challenge of mixing and carry-over between new and old sweat. **Figure 2C** shows an example of sweat collection microfluidic devices, coupled with glucose and lactate biosensors [20]. This offers wearable effective continuous sweat sampling and flow electroanalysis.

Furthermore, minimally invasive microneedles for continuous glucose monitoring have been demonstrated. For example, a GOx/tetrathiafulvalene microneedlebased amperometric sensor (~1.2 mm needle height) could be used for in vivo studies [21]. The data were also validated with the finger-prick technique, indicating a promising alternative for on-skin analysis. In addition, a minimally-invasive microneedle-based potentiometric sensor for tracking β-lactam antibiotic concentrations in vivo and real time was demonstrated **Figure 2C** [22]. This example represents a possibility to tailor individual therapy with the optimal efficacy.

Moreover, reading several parameters can complete a clear picture of individual health. A fully integrated sensor array for sweat analysis was demonstrated (**Figure 2E**) [23]. These integrated sensors can monitor information of glucose, lactate, electrolytes (e.g., sodium and potassium ions), and temperature. The temperature sensor is also helpful to standardize the biosensing amperometric response. Furthermore, in order to apply the biosensor glucose device for health management, a transdermal closed-loop drug delivery integrated with a sweat-based glucose electrochemical sensor was demonstrated (**Figure 2F**) [24]. The sense-treat concept aimed to give feedback of transdermal administration of type 2 diabetes drugs in response to the glucose level. This idea represents a possible opportunity to overcome insulin overtreatment, helping patients to maintain their homeostasis.

2.2 Enzyme-based electrochemical power sources

BFCs are energy-conversion devices that utilize biocatalysts to convert chemical energy into electricity. For wearable electronics, the need to anatomically power sources has attracted many research groups to develop a BFC, as a "green" energy-harvesting alternative, in order to extract energy from metabolites present in biofluids, such as perspiration. Since glucose, lactate, and oxygen are present in physiological fluids, in general, a majority of wearable enzymatic BFCs rely on (1) the generation of electrons from glucose or lactate biofuels and (2) the electron reduction by oxidants (such as oxygen). **Figure 1C** shows a typical example of a glucose/O_2 BFC. In principle, a glucose BFC uses GOx, functionalized on the bioanode, to catalyze the glucose oxidation reaction to generate electrons. After this oxidation process, these harvested electrons are driven through an external circuit to the biocathode compartment where such electrons are accepted by oxidant molecule (commonly O_2) and, eventually, generate complete electrical work. In addition to

Pt-based catalysts, multicopper oxidases such as laccase, bilirubin oxidase, and polyphenol oxidase are commonly used for electrocatalyzing oxygen-reduction reaction (ORR) in the BFC cathode [25].

Enzymatic BFCs represent an interesting alternative due to their unique advantages, such as outstanding selectivity and behaviors of enzymes. Unlike most traditional inorganic catalyst-based fuel cells, which require harsh conditions (such as acidic conditions or high temperatures ranging from 45°C to more than 100°C), the enzyme-based BFC can operate under mild conditions (20–40°C at neutral pH). Moreover, non-specific catalyst-based fuel cells require to separate anode and cathode chambers by a thin membrane. Unfortunately, this common use of separation membrane between the anode and the cathode compartments will be unsatisfactory for skin-worn miniaturized devices. Thanks to the nature of enzymes, utilizing high specificity of enzymatic catalysis can obviate this membrane requirement, facilitating the fabrication and applications [26]. In addition, enzyme-based BFCs can operate selectively in complex biofluids.

Interestingly, BFCs also offer opportunities to design self-powered biosensors (**Figure 1D**). For example, the power is proportional to the concentration of the fuel (also acting as analyte); self-powered output itself can determine the level of the target. This offers opportunities to eliminate external energy sources for powering potentiostat and signaling systems [9].

An initial concept integrating enzymatic BFCs with skin-worn technologies represented an exciting way to scavenge bioenergy available in human perspiration (**Figure 3A**). This demonstrated the first epidermal tattoo-based BFC that converted sweat lactate biofuel and oxygen into electricity [27]. The lactate oxidation by LOx electrocatalyzation was mediated by tetrathiafulvalene on the carbon nanotube (CNT)-based anode, while electroreduction on the oxygen-reduction cathode relies on Pt black catalyst. This system facilitates mediated oxidation of lactate at −0.1 V with a peak potential of 0.14 V (versus Ag/AgCl). This low anodic onset potential indicates the efficient electron-donor-acceptor TTF/CNT. The successful on-body test displayed a power up to 70 μW cm^{-2}. This idea was also established on fabrics and could power a light-emitting diode with an integrated DC-DC converter [28].

Mechanical stability has been the focus in the development of the next-generation of skin-worn BFCs due to the multiplex mechanical movements experienced *in vivo*. In order to minimize cracking of the device and maintain good electrochemical performance, screen-printable stretchable inks and judicious stretchable design have been engineered (**Figure 3B**) [29]. Combining additional degrees of stretchability with intrinsic mechanical resiliency of soft CNT/polyurethane (PU) composite offers the desirable stretchable platform. The BFC was then functionalized on the soft electrodes, allowing good mechanical stability. This holds promise applications for on-body bioenergy fields wherein resilience toward mechanical distortions is compulsory.

In addition to energy-conversion applications, BFCs can be applied further as another significant tool for wearable bioelectronics. Enzymatic BFC can serve as self-sustainable biosensors (without an extra powering device). In order to expand the spectrum of BFC applications for on-skin electroanalytical chemistry, the pioneering stretchable textile-based BFCs that can act as self-powered was demonstrated (**Figure 3C**) [30]. These biodevices can deliver two key functions: (1) harvesting electrical power from sweat glucose and lactate and (2) displaying signals of such metabolites. Extracted bioenergy from the wearer's sweat can directly indicate the metabolite levels. Sock-based biodevices were successfully demonstrated on human subjects, representing a promising concept for modern wearable self-powered biosensors.

Maximizing the loading amount of active enzyme, mediator, and conductive materials can improve the power performance of BFCs. The high amount of such active materials can be packed by a compress. However, this strategy will affect mechanical softness. Therefore, further engineering was to fabricate island-bridge assemblies merging the high enzyme loading packed islands with stretchable serpentine bridges [34]. This combination offered a soft bioelectronic skin for harvesting a good power density of 1.2 mW cm^{-2}. This energy was sufficient to power a Bluetooth Low Energy (BLE) radio integrated with a DC-DC converter.

Recently, additional efforts have been made to scavenge, improve, and store energy by hybridizing textile-based energy conversion with energy storage devices (BFCs and supercapacitors, respectively) (**Figure 3E**) [31]. The on-body demonstration showed that after perspiring, the supercapacitor could be charged by the BFC energy and reach a stable 0.4 V output.

Furthermore, a photoelectric BFC was developed to convert external light and chemical energy from wearer's perspiration into electrical energy (**Figure 3D**) [32]. The anode relied on a LOx/Meldola's blue/buckypaper electrode, while the photocathode
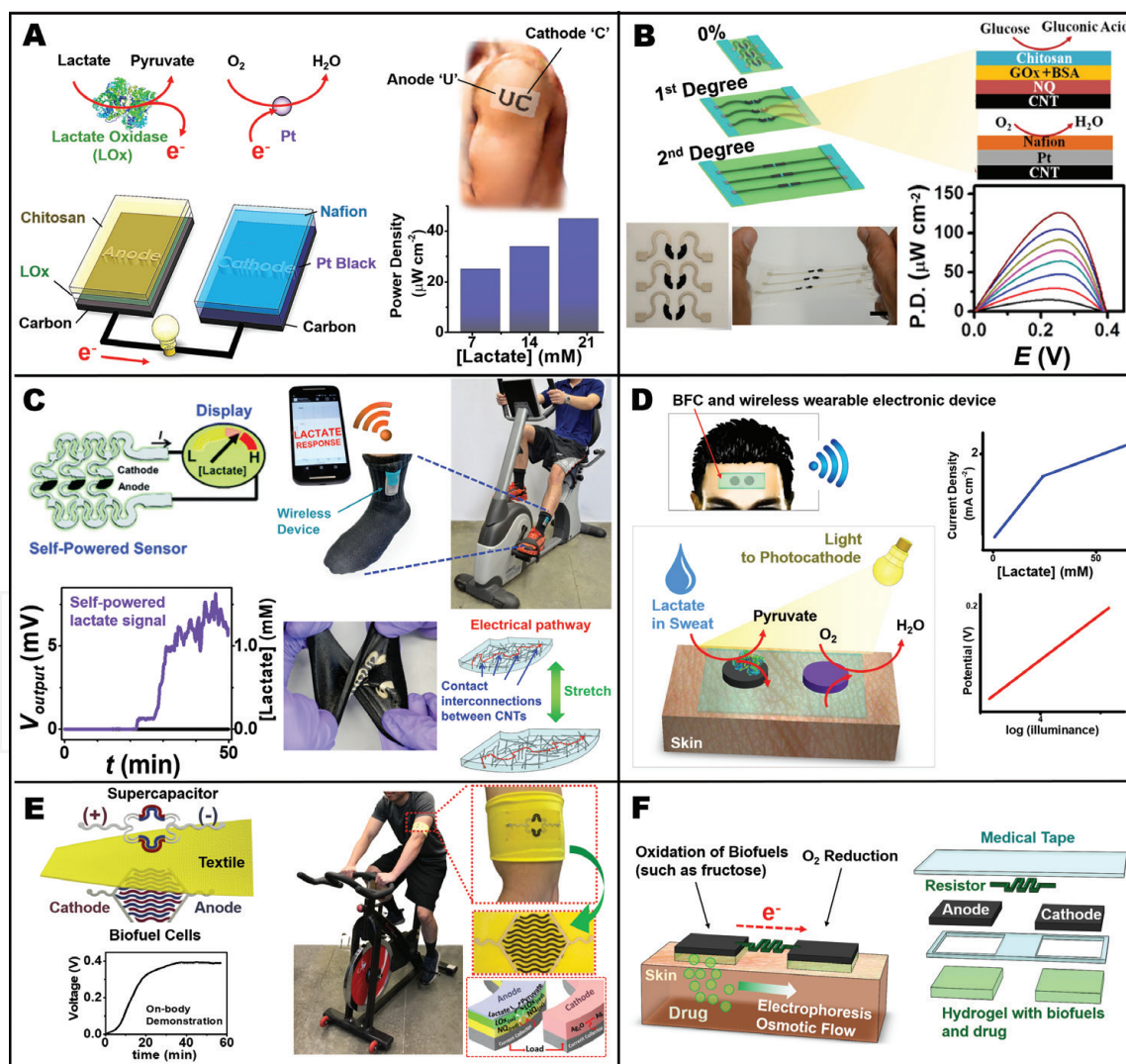

Figure 3.
Skin-worn BFCs and self-powered sensors. (A) Epidermal tattoo-based lactate BFCs. (B) Stretchable glucose BFCs [29]. Adapted with permission from ref [29]. Copyright 2016 American Chemical Society. (C) Stretchable textile-based BFCs acting as self-powered biosensors [30]. Adapted with permission from ref [30]. Copyright 2016 The Royal Society of Chemistry. (D) Photoelectric BFCs. (E) Textile-based BFC-supercapacitor hybrid devices [31]. Adapted with permission from ref [31]. Copyright 2018 The Royal Society of Chemistry. (F) Built-in BFCs with transdermal iontophoresis patches.

relied on an organic polyterthiophene semiconductor, which drove a reduction reaction under illumination (wavelengths of 350 nm to over 600 nm). This system presented an attractive example of on-skin autonomous power sources and sensors.

Additional efforts have been made to explore new biomedical applications of BFCs. **Figure 3F** shows an integrated fructose/O_2 BFC patch that was conjugated with transdermal iontophoresis [33]. The current generated by the BFC was used to drive an osmotic flow from the anode to the cathode, resulting in the net ionic movement of small-molecule drug into the skin. The level of transdermal current to control the drug administration could be adjusted by connecting a thin poly(3,4-ethylenedioxythiophene)/PU resistor of a programmable resistance value.

3. Challenges and possible solutions

3.1 Mechanical properties

Young's modulus of the human skin is in a range of 10–500 kPa [35, 36], while the moduli of common electronic materials, such as silicon and gold, are much higher (high GPa), indicating significant mechanical mismatch when integrating with the skin. Therefore, functionalities of non-stretchable electrodes will deteriorate after multiplex deformations commonly experienced by daily life activities. Furthermore, such rigidity and bulkiness of traditional devices also restrict the wearability and comfortability [14]. Non-compliant electrochemical devices will limit continuous long-term functions due to cracking and increasing of material resistance. This increasing of resistivity, which opposes the current flow in bioelectronics, causes poor electron communication at the enzyme-electrode interface.

This major challenge of skin-integrated electronics can be addressed by exploring stretchable materials which display mechanical properties in a similar range of skin's modulus. One approach is using polymers due to their low mechanical toughness. For example, conducting materials with high moduli can be blended with soft polydimethylsiloxane or Ecoflex materials (Young's moduli of 0.4–3.5 MPa and 125 kPa, respectively) in order to tune the mechanical properties while keeping good electrochemical functions [37]. CNT-based materials, which are powerful for electrochemical devices [38], are used to combine with soft elastomers, such as PU and styrene-butadiene-styrene (SBS) [29, 39]. PU and SBS composites have moduli of ~700–800 kPa. As shown in **Figure 3C**, CNT filler (with the high-aspect ratio ~1300) was combined with PU [30], achieving stretchable conductive electrode materials. The percolation of dispersed CNTs can facilitate the electric flow in stretchable bioelectronics. Combining the intrinsic stretchability of this engineered inks with the structural stretchability of the serpentine design allows the device to tolerate strains as high as 500% with a small effect on its electrochemical performance [29]. This concept can be expanded by adding new functionalities into electrodes. For example, platinum-decorated graphite was mixed with PU to obtain stretchable electrocatalytic materials, allowing the fabrication of stretchable electrodes for glucose biosensors [40].

3.2 Powering wearable devices

Growing demand of wearable technologies has stimulated the need of the development of viable energy sources. The lack of anatomically power sources becomes a key bottleneck for the progress in wearable bioelectronics. Skin-worn bioelectronics mandates the compliant and efficient energy sources to supply multitasks, including

sensing and data communication. In addition to developing low-power-consuming electronic microelectronics [9, 41], there is an increasing interest in advancing bioenergy-harvesting devices. Enzymatic BFCs are attractive self-sustainable energy devices to meet this growing energy demand. For example, 0.3-V complementary metal-oxide-semiconductor (CMOS) wireless glucose or lactate biosensing systems, which consumed power of ~1.2 µW, could be powered by BFCs [9]. Nevertheless, several applications of enzymatic BFCs still have some challenges, such as low-power output. The major challenge in enzymatic BFC is faced by the electrical "wiring" of enzymes with electrodes. The difficulty of electrical wiring, referring to electron transfer, and their possible solutions will be detailed in Section 3.3.

Compared with traditional fuel cells, enzymatic BFCs are challenging due to their multicomponent including redox potentials of enzyme, cofactor, and mediator. This results in the typical unwanted deviation of open-circuit voltages (OCV) from their theoretical maximum values, referring to "cell voltage losses." The redox potential for electrocatalytic oxidation at the bioanode required to be higher than that of the biocathode for reduction reaction in order to deliver a sufficient electromotive force for electron transfer between enzyme active site and mediator. The voltage difference between the formal redox potentials ($E°'$) of redox enzyme cofactors in the active sites, in the anode and cathode, will govern the maximum cell voltage. Parameters, including redox potential of mediator and cofactor redox potential in the enzyme, can influence the resulting potential output of BFCs. Therefore, the mediator should be carefully chosen. For example, ferrocene derivatives coimmobilized with GOx at a graphite electrode can be used for glucose sensors [42]. Nevertheless, ferrocene derivatives display high redox potentials (0.1–0.4 V versus SCE); these will cause cell voltage losses in the GOx-based BFC if they are used as anode mediators. It should be noted that the difference between the redox potentials of the enzymes wired at the anode and the cathode determines the cell voltage. An example of a successful anode mediator used in skin-worn BFCs is 1,4-naphthoquinone [30]. This quinone compound is also almost insoluble in cold water, preventing leaching during on-body operations. One challenge of using GOx on the anode is the O_2 competition with a mediator, decreasing the oxidation current on the bioanode. Moreover, O_2 competitive reaction on the anode can produce H_2O_2. This by-product can inhibit GOx activity and decrease the overall BFC performance. Therefore, catalase should be cofunctionalized to the bioanode to diminish the undesirable H_2O_2 [43].

A single-enzyme BFC can usually convert only a partial portion of biochemical energy, resulting in low current output. For instance, wearable BFCs, such as for harvesting energy from lactate sweat, commonly employ a single enzyme-based bioanode, catalyzing the oxidation of lactate to pyruvate, which only harvests two electrons. In other words, they utilize only a portion of the biofuel energy and leave most of the energy in the oxidized product. Therefore, it is interesting to harvest the total of 12 electrons in order to maximize the energy-conversion efficiency. A potential solution is to design an enzyme cascade system for complete oxidation of lactate fuel. For example, the bioinspired multienzyme catalytic cascade could complete the metabolic cycle, successfully enhancing net BFC power [44].

Furthermore, in order to optimize the current output, diffusion and enzyme loading should be enhanced. The engineering of specific enzyme activity and three-dimensional structure of enzymatic electrodes should be explored.

3.3 Enzyme-related aspects

The selection of enzymes is a primary subject which should be discussed. Enzymes must be selected by considering their particular reactions to target

analytes or biofuels for electroanalytical monitoring and energy harvesting, respectively. One of the most predominant enzymes used to develop wearable bioelectronics is GOx from *Aspergillus niger*. It represents an example of commercially available biocatalyst that has good stability, substrate specificity, and electron turnover rate [3, 4, 45]. It is a powerful biorecognition element for glucose biosensors, the most widely interesting devices for diabetes health management. As shown in **Figure 4** (A–C), the enzyme is immobilized on the electrode, establishing a biosensor. GOx contains two 80 kDa subunits. Each holds a tightly bound flavin adenine dinucleotide (FAD) cofactor, the important redox center which has a redox potential -0.32 V (vs Ag/AgCl) at pH 7. This redox center is crucial to transfer electrons and specifically oxidize β-D-glucose to gluconolactone. However, this FAD is shielded by the protein and a glycan structure, hindering electron exchange at the enzyme-electrode interface. Inevitably, this requires research efforts to address this roadblock [46, 47]. FAD plays an important role as a common cofactor for glucose oxidation biocatalysis. The redox process for $FAD/FADH_2$, involving two electrons, is shown in **Figure 4D**, where the R group represents adenosine diphosphate and ribitol connected with the flavin. However, it is O_2-dependent; accordingly, O_2 fluctuations can vary the performance of this type of oxidase-based bioelectronics. Although alternative O_2-independent electrodes utilized NAD-dependent electrodes can be used, they require a diffusional cofactor, not simple for wearable applications. Hence, FAD-dependent dehydrogenases are becoming interesting choices since they are O_2-independent and do not depend on diffusional mediators [48, 49].

The first generation of biosensors relies on quantifying O_2 generation or H_2O_2 depletion (**Figure 4A**). This leads to key drawbacks, such as low dynamic range, dependency to oxygen fluctuations, and interfering effects. For instance, for glucose amperometric sensors, the detection of H_2O_2 at common first-generation electrodes needs the high applied detection potential where interfering compounds existing in sweat, e.g., ascorbic acid, uric acid, and some drugs, are also electroactive. Lowering the applied potential for the detection is a strategy to minimize
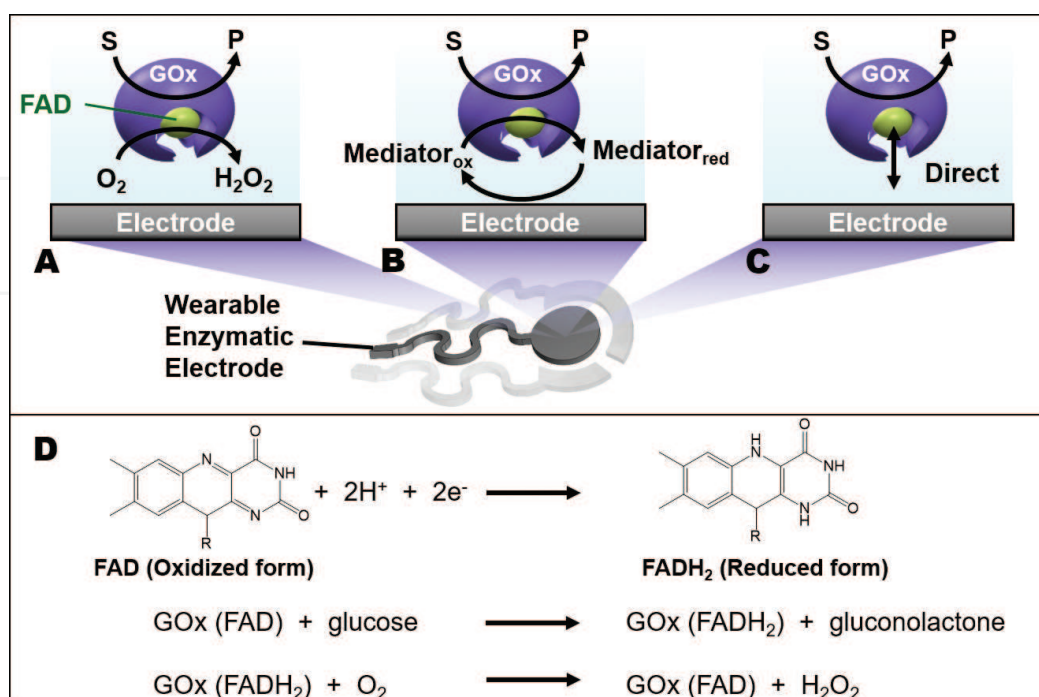

Figure 4.
Principles of interfacing the enzyme, such as glucose oxidase (GOx), with the electrode. Different generations of strategies (A–C: first, second, and third generations) are illustrated. (D) Reactions involving the glucose oxidase biocatalyst.

such electroactive interferences. One approach is to incorporate electrocatalysts in wearable electrodes, such as PB or Pt [17, 40]. This offers low-potential detection of H_2O_2 to mitigate interference effects.

Furthermore, researchers have developed two strategies to wire enzymes to the electrode interface (**Figure 4B** and **C**). These include (1) mediated electron transfer (MET) and (2) direct electron transfer (this may refer to mediatorless electron transfer between the enzyme and the electrode). Such new tactics are not only useful for enzymatic biosensors but also for enzymatic BFCs which also involve bioelectrocatalysis.

First, the MET strategy utilizes a redox mediator, acting as an electron-shuttle assistant between the enzymatic active center and the electrode. The substrate level, such as glucose, can then be monitored by the redox process of the mediator. This results in the independence of oxygen and mitigating the interfering signals due to the operation at low potentials. The first consideration in electrically wiring the enzyme with the electrode is the choice of the mediator that should be close to the redox potential of the active center of the enzyme to facilitate efficient electron communication between the enzyme and the conductive electrode surface. In particular, for enzymatic BFCs, the selection of mediators is crucial to positively control the cell voltage and enhance heterogeneous electron transfer to the order of a homogeneous transfer [50]. However, challenges of using mediators, particularly for BFCs, are their stability and deviated cell voltage. In addition, biocompatibility is highly vital for skin-worn applications. In spite of the assistance of electron shuttle by redox mediators, major concerns are their biocompatibility. One possible solution is employing nanomaterials or highly biocompatible catalysts. For example, mushroom/plant extracts could be used to obtain efficient "green" bioelectrocatalytic reactions for ethanol BFCs [51].

Second, direct electron transfer is an ideal goal of electrical wiring. It can be achieved by employing nanomaterials which suggest the direct electron transfer between enzyme active site and electrode. This wiring strategy is based on the shortening of the electronic contact of the enzyme and electrode (a short distance of ~1.5 nm) where the redox center of the enzyme can be regenerated directly by the electrode [52]. Therefore, this strategy can maximize the performance of bioelectronics. The engineering needs to consider the position of the active site inside the protecting protein and the conformation of the protein in order to wire the conducting materials with the redox center. This still remains the most challenging topic.

Several variables also affect the response nature of enzyme bioelectronics. Consideration of the fundamental theory of their functions will help to improve their performances. A key well-known model of enzyme behaviors is Michaelis-Menten kinetics, $V_0 = V_{max}\frac{[S]}{K_m + [S]}$, where V_0, V_{max}, K_m, and *[S]* are the initial velocity of the reaction, the maximal rate of the reaction, the Michaelis-Menten constant, and the concentration of the substrate, respectively. In general, it is desirable to engineer the biointerface electrode system to obtain high V_m and low K_m (good affinity). However, dynamic range is also a crucial characteristic for wearable biosensors. Traditionally, dilution or preconcentration can be used to adjust the level of the target to be fit in the linear range of the sensor; nonetheless, manipulating such processes for on-skin applications is sophisticated. Therefore, diffusion-limiting membranes may be a useful solution to tune the dynamic range. The linear range can be extended by coating a thin membrane over an active enzyme layer since the sensor response is controlled by the analyte diffusion and not by the nonlinear characteristic of enzyme kinetics. Nevertheless, it should be noted that coating may lower the sensitivity and cause slow response time.

In addition, extra membranes can be a biocompatible barrier to address challenges from biofouling and interferents, especially when electrochemical operations are made in real matrices, samples, such as sweat. A perfluorinated sulfonated membrane (Nafion®) is an example membrane, which is also easy to drop-cast. This coating membrane can protect the enzymatic layer and also prevent anionic interferents, such as ascorbate [53].

Shelf life and operational stabilities of enzymatic electrodes are among the most critical challenges. The enzyme and active materials, such as mediators, can also leach during operations. Extensive studies have been made to improve enzyme bioelectrodes, such as by crosslinking hydrogels in the presence of the enzyme [54, 55]. Such crosslinking can entrap the enzyme to be more stable; moreover, this way enhances the loading of the enzyme, while the three-dimensional structure can facilitate the transport of analytes or biofuels, improving bioelectrode functions. Nevertheless, crosslinking enzyme or covalent binding of the enzyme can change the conformation of the enzyme and thus affect the activity [56]. Furthermore, one alternative to stabilize the enzyme electrode is the addition of stabilizers, such as polyelectrolytes, dextrans, glycerol, polyethyleneimine, and hydrophobic oils [57–59]. For instance, hydrophobic mineral oil or silicone grease can be used to minimize enzyme denaturation [58, 59]. The pasting liquid helps to lower protein mobility, maintain conformational rigidity of enzymes, and barrier to hydronium ions from acid environments. This strategy can stabilize many enzymes, such as GOx, LOx, AOx, horseradish peroxidase, amino acid oxidase, and polyphenol oxidase.

Increasing enzyme loading can also improve the performance of biocatalytic devices. Employing high surface nanomaterials is useful to enhance the surface loading of the target catalyst. A graphene-based electrode is a good example platform to offer a high enzyme loading (1.1 nmol cm^{-2}); in addition, it offers a fast heterogeneous electron transfer rate (k_s) of 2.8 s^{-1} [60]. Moreover, CNTs, which have high conductivity and specific surface, represent an outstanding candidate nanomaterial for electrochemical wiring [38, 61]. The thin nanoscale structure can intimately incorporate with the active enzyme. Adsorption of GOx on CNTs provides the apparent k_s, of 1.5 s^{-1} [62]. The k_s of GOx at the hybrid biocomposite can be as high as 11.2 s^{-1} [63]. Therefore, mediatorless bioelectrodes with excellent electron transfer could be demonstrated. Their high three-dimensional architecture also offers an enhanced loading of enzyme and/or redox mediator immobilizations. As a result, this can enlarge the current output from the biosensor or BFCs. Importantly, for BFCs, the maximized OCV and current density could be observed [43]. This BFC consists of a GOx/catalase/CNT bioanode and laccase/CNT biocathode without additional mediators. The CNT/enzyme matrix was compressed together under high hydraulic pressure (10 kN). The resulting output in an air-saturated electrolyte (200 mM glucose in 0.2 M phosphate buffer solution, pH 7 at room temperature) after 3 days displayed a high maximum OCV of 1 V. Note that the GOx/catalase/CNT bioanode and the laccase/CNT biocathode showed OCV values of −0.35 and +0.6 V, respectively.

Importantly, biofluids from the skin (such as sweat and extracted interstitial fluids) contain a variety of chemicals that can inhibit enzyme activity, reflecting challenges in biosensing and BFC functions in real-time on-body applications. For instance, heavy metals can be found in sweat as the body expels chemicals or balances the charges. One example is Cu^{2+} which has been reported as an inhibitor to deactivate the enzyme. The Cu^{2+} in sweat can be in a range of 1.6–16 µM [11]. 0.1 µM Cu^{2+} could decrease the OCV value of the glucose BFC [64]. However, this enzyme-inhibitor electrochemical behavior is analytically attractive toward the development of self-powered biosensors, such as for direct heavy metal screening

or indirect cysteine monitoring. For example, cysteine prefers to bind with Cu^{2+} via the Cu-S bond; this superior conjugation between cysteine and Cu^{2+} removes metal ions from the bioanode, consequently turning on the OCV.

3.4 Effects of oxygen fluctuations on electrochemical performances

Since the O_2 level in biofluids may vary, first-generation biosensors, employing O_2-dependent mechanism, are subject to inaccuracy. This issue can be addressed by using fluorocarbon pasting liquids to supply internal O_2 [65]. Using redox mediator as a second-generation sensor is another way to eliminate this error. Furthermore, FAD-dependent glucose dehydrogenase is an option to address O_2-dependent problems due to its O_2-insensitive nature, compared with GOx [49]. In addition, because of the high rate of homogeneous electron transfer rate between GOx and oxygen, GOx prefers to transfer electrons to oxygen rather than to the electrode, causing undesirable O_2 competition effect [66]. Moreover, for BFCs and self-powered sensors, the commonly used ORR cathode may cause the error under anaerobic conditions. The use of Ag_2O/Ag redox cathode, which does not depend on ORR, can be used to operate BFCs, mitigating the possible O_2 errors [30, 67]. Note that the reduction potential of Ag_2O/Ag (0.342 V vs. SHE) is close to that of O_2/OH^- (0.401 V vs. SHE) at pH 7. Moreover, using O_2-rich cathode is another possible option to mitigate O_2-deficit effects [68].

3.5 On-skin biofluid extraction: electrical-based approaches

Each person has 2.03 million sweat glands; sweat gland densities vary broadly across the skin surface and subjects, ranging from 16 to 530 glands cm^{-2} [11, 13, 69]. Normally, during exercise, sweat can be secreted around 20 nL $gland^{-1}$ min^{-1} [11]. For example, the forehead or arm can generate sweat around 3 µL cm^{-2} or even lower. The fluctuation of sweat rate is also related to numerous factors, such as activity intensity and hydration level. Therefore, the limited volume of sweat causes a challenge in sweat analysis and operations. This leads to the development of miniaturized skin-worn electrochemical devices that can be practical in such small dead volume. For instance, the textile-based energy-harvesting BFC requires sweat volume per area of 40 µL cm^{-2} to deliver steady outputs [31]. Designing a capillary chamber is a possible route for low-volume electroanalytical systems [70].

In addition to a passive way to collect sweat, one strategy is an active electrical-based approach, called "iontophoresis" [71, 72]. This active strategy offers
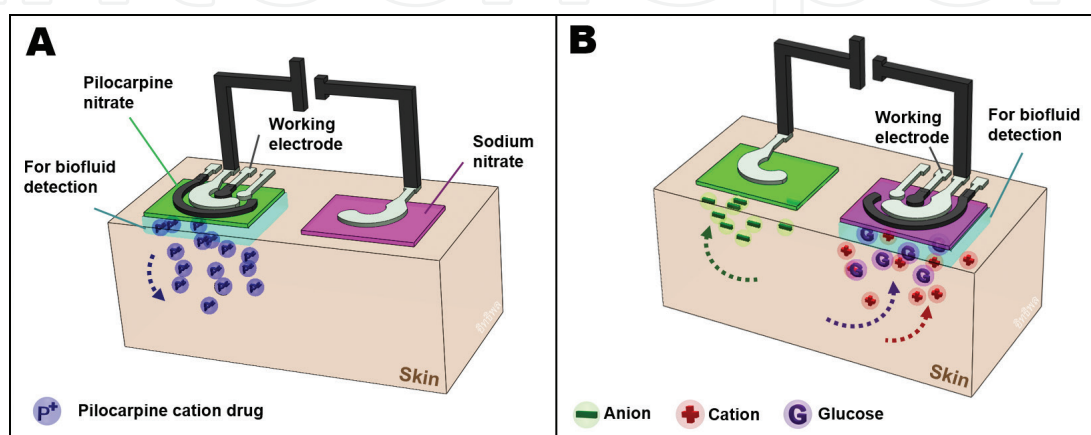

Figure 5.
Electrical-based strategies using iontophoretic electrodes to extract biofluids, including (A) pilocarpine iontophoresis and (B) reversed iontophoresis.

on-demand sweat generation as the device can be placed to a local skin target. There are two main approaches to extract sweat: (1) iontophoresis with pilocarpine drug and (2) reversed iontophoresis without the drug. These are attractive routes for continuous sweat analysis.

First, pilocarpine iontophoresis can be used to stimulate the sweat. In principle, a small electrical current is applied to enable the pilocarpine administration across the epidermis as illustrated in **Figure 5A**. For example, the tattoo-based enzymatic alcohol sensor consists of a pair of electrodes located in contact with the skin surface. Small constant current ($0.2 \, \text{mA cm}^{-2}$) was applied through the cryogel material containing pilocarpine at the anode (positive) iontophoretic side [18]. The applied electrical force will push the pilocarpine drug, which possesses a large positive charge, to eventually enter into the skin. Such transdermal drug delivery of pilocarpine can induce the local sweat, sufficient for the subsequent electro-chemical detection. In addition, interstitial fluid (ISF) located under the skin can be extracted. Without this iontophoretic strategy, it is challenging to access ISF through wearable technology.

Second, the reversed iontophoresis without pilocarpine drug can be used to extract relevant analytes, such as glucose [17]. For instance, as presented in **Figure 5B**, a current ($0.2 \, \text{mA cm}^{-2}$) is applied to extract glucose in ISF. During the reverse iontophoresis process, glucose is pulled out at the negative iontophoretic compartment. Even though glucose holds no charge, the inherent permiselective characteristic of the skin prefers to transport positive species, allowing such glucose extraction. Applying electric field on mobile electric charge can cause Coulombic force, leading to a net convective flow in the skin from the anode to cathode direction. Accordingly, dissolved analytes (e.g., glucose) are also moved toward the cathode where they can be extracted and monitored. Therefore, the glucose amperometric working electrode, adjacent to the cathodic iontophoretic side, can detect the glucose level from the extracted sample.

4. Conclusions and future prospects

This chapter has reviewed some examples of new trends of skin-worn enzyme-based electrochemical systems, focusing on biosensors, BFC, and self-powered sensors. The existing systems provide significant advances toward the painless and point-of-care applications and personalized electrochemical biodevices, which was not possible without such new biodevices. However, researchers still face many challenges, such as electrochemistry, electrical wiring of enzymes, enzyme behaviors, the fabrication of stretchable electrodes, O_2 fluctuations in biofluids, interferences, and difficulty in sweat extraction. Moreover, the workability and reliability of biodevices can be limited due to the limited fluctuating and volume of biofluids. In order to avoid frequent recalibrations, the stability of biodevices or self-calibration systems are also important. Precise electrochemical functions for on-skin applications are still very challenging. Therefore, it is required careful attention to address all challenges in order to advance such wearable technologies.

Although main skin-worn BFCs have been driven by glucose and lactate fuels, it is interesting to explore new opportunities, such as from alcohol-based BFCs, where the bioanode can be functionalized with alcohol dehydrogenases. Future efforts may be made to expand the spectrum of current concepts. New integrated devices can be achieved by designing multifunctional sensors that can provide informative series of personalized data. This will require the incorporation of big-data analysis and Internet of things (IoT) to build up integrated networks and personalized baselines of each wearer. Big data collected from networks and individuals can

then warn the user whether the body is in a healthy and equilibrium state or not. It is expected that developing new electrochemical biodevices will eventually track "fingerprints" of various pathologies and disorders. This aims toward wearable systems for early disease diagnosis. Moreover, full closed-loop concepts such as biocomputing logic gate, sensing, and therapeutic systems can also be further exploited in the integration of biosensors, BFCs, and drug delivery devices, in order to obtain both diagnostic and therapeutic applications. The next success of wearable biodevices needs the hybrid of multidiscipline, including physiological medicine, electronics, electrochemistry, bio- and nanoengineering, and computer science. These continued collaborative efforts will open fantastic opportunities for addressing current challenges and step further to create novel wearable devices and acquire comprehensive big data. Ultimately, it is expected that innovative wearable electrochemical technologies and new findings will contribute to revolutionizing diverse personalized wearables and biomedical applications.

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