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Perspective—Challenges in Developing Wearable Electrochemical Sensors for Longitudinal Health Monitoring

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Wearable electrochemical sensors have the potential to overcome the problem of infrequent clinical visits that leads to transient events of potential diagnostic importance being unduly overlooked. The promise of real-time, personalized health care has driven multidisciplinary work on fabricating various forms of wearable sensors. Although remarkable advances in device form factor and integrated circuit design have been achieved, notable hurdles, such as shelf life, reuseability, flex and sweat resistance, and longitudinal performance, remain unaddressed. This perspective seeks to summarize major advances in current wearable electrochemical sensors and to highlight the most pressing challenges that will benefit from collective research endeavors.

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With the increasing costs of healthcare and concomitant rise of sensors and actuators connected by wireless networks (the “Internet of Things”), personalized physiological monitoring using wearable devices has received great attention.¹ Wearable chemical sensors, functioning at a critical intermediary interface, are regarded as attractive alternatives to current bulky analytical instruments. Their potential to monitor and respond to both the wearer and his or her surrounding environment in real time can overcome the problem of infrequent clinical visits that may fail to detect transient events of important diagnostic importance.² Although this promise of real-time, personalized health care has rapidly driven the development of many proof-of-concept wearable electrochemical sensors, devices that present reliable molecular-level information over long monitoring periods are still far off.

This short perspective seeks to summarize recent advances in creating wearable electrochemical sensors for longitudinal health monitoring and to highlight the most pressing challenges in this field that will benefit from collective research endeavors. The article is organized as follows: first, we provide a quick inventory of biomarkers and small-molecules in body fluids that are important targets for wearable electrochemical sensors, then we dissect the typical device layout of current flexible chemical sensors, and, finally, we discuss the limitations of known work and provide a critical perspective for future development.

Targets for wearable electrochemical sensing systems.—Biomarkers and small-molecule targets in common body fluids, especially in blood and sweat, offer important information about human health status and body performance. Further, the concentration of a number of metabolites, ions and proteins in sweat, saliva, tears and urine have been established to track linearly with their respective concentrations in blood,³ meaning that real-time chemical monitoring of these targets using noninvasive wearable sensors is conceptually feasible.

Sweat, which is a representative biofluid containing a variety of biomarkers has particularly gained interest.⁴ Also, xenobiotics created by environmental factors may also get trapped on the skin surface and subsequently dissolved in sweat. Since human skin provides a broad platform for collecting and analyzing sweat, skin-mounted sensors (temporary “tattoos”, examples are listed in

Table I) enable a variety of chemical sensing possibilities. Although conventional approaches to obtain sweat samples involve intense exercise or high-temperature environments that induce sweating, drugs like pilocarpine,⁵ methacholine, acetylcholine,⁶ can also be used to chemically stimulate eccrine sweat glands and comfortably collect the necessary sweat samples under natural settings. Physical stimuli, for example, exposing skin to aqueous ethanol, can also help to collect sweat.⁷

Device categories.—To make a sensor for real-time and on-body sweat analysis, besides the fundamental requirements of sensitivity and selectivity, comfortability and portability are also critical. Nowadays, many studies focus on creating wearable sensors containing electrochemical transducers due to their instrumental simplicity and ready miniaturization capability. Moreover, electrochemical techniques are powerful tools owing to their high performance, low cost and compatibility with near-field communication, which enables final readouts on ubiquitous mobile phones.

In terms of the electrochemical sensor, the input (a physical or chemical event) occurs at the sensor’s surface and is translated into an electrical signal by transducer components (target-specific receptors or recognition elements on the working electrode). Typically, these signals are also calibrated using either an external standard or an internal reference electrode. Depending on the nature of their final output, electrochemical sensors are divided into three major types as listed in Table II.

Current Status of Wearable Sensing Systems

To date, mostly skin-mounted electrochemical sensors (tattoos) have been developed, which display notable sensitivity and selectivity. Human skin is very anisotropic and viscoelastic, with a Young’s modulus ranging from 57 to 140 MPa and a 25%–80% breaking elongation.³¹ Generally speaking, materials that are compatible with the unique mechanical strength of human skin can be divided into natural and synthetic classes. Common natural materials are cellulose-based (cotton) or protein-based (wool or silk), while synthetic materials are polymers exhibiting elastic properties, including PET, poly(amide)s, poly(acrylonitrile), and poly(propylene) (PP).³¹

The working electrode is a key component in all known iterations of smart tattoos. The broadly defined structure of the working electrode in a wearable sensor consists of a flexible substrate, a conducting material, a recognition agent, and a biocompatible protective layer. Flexible substrates, such as poly(vinyl alcohol) (PVA) based temporary tattoos, silicones (e.g. PDMS, Ecoflex,

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Table I. Known targets for wearable sensors.

Category	Biomarker	Application	References
Electrolytes	Sodium, Potassium; Chloride	Identify cystic fibrosis; Monitor hydration	6, 8
Small Molecules	Glucose; Urea, Creatinine; Uric acid; Cortisol; Ethanol, Mephedrone;	Diabetes screening; Kidney failure evaluation; Wound healing monitoring; Stress evaluation; Drug dosage control	9, 10–15
Xenobiotics	Heavy metals; Drug residues; Cosmetic residues	Product safety evaluation; Forensic chemistry	16,17, 18
Body odor	Ammonia, Acetic acid;	Athletic performance	17, 18

Solaris) and inert plastics (e.g. PET, PEN), exhibit elastic properties similar to human skin and have therefore been used as substrates for skin-mounted biosensors.¹⁶

Two basic steps are needed to realize functional sensors on these flexible substrates. First, finding a proper conductive base and recognition agent to ensure good chemical response. Then the second step is to develop a method to load these materials onto the flexible substrate that ensures the active layer will not be delaminated with varying skin tension. Although forming films of conventional conductive materials, such as metals or conductive carbon, on flexible substrates can be challenging due to their hardness or brittle nature, new materials and processing methods have been developed to address this problem in recent years. In terms of metals, designing serpentine conducting lines with periodicities guided by theoretical simulations can increase their stretchability and bendability.³¹ Another way is to leverage solution-processible inks of nanostructured conductors, such as metal nanoparticles and graphene.¹⁷ These inks can be easily printed onto flexible substrates via methods like screen-printing, inkjet printing, gravure painting³² and doctor blading.⁵ Also, due to their tunable conductivity and highly ordered 3D structures, conductive polymers are another powerful alternative. Electrosynthesis, in situ solution-based polymerization and reactive vapor deposition have all been applied to create conducting polymer films on flexible substrates.²⁷

Recognition agents, namely bio-receptors, vary depending on the target analyte. In terms of potentiometric and impedimetric sensors where no current flows in the system, binding and recognition sites are provided by commercial ionophores,²³ hydrogel templates, self-assembled monolayers,⁹ or antibodies (for ion, charged gas and protein sensing).³³ On the other hand, amperometric sensors that are based on enzymatic redox reactions usually require more complex fabrication methods. Due to the hierarchical structures and intrinsically-high reduction potentials of enzymes,⁴ electron mediators are sometimes needed to facilitate electron transfer from buried enzyme pockets to the electrode, and to lower the applied working potential during sensing.³⁴ Prussian blue (PB) and tetrathiafulvalene (TTF)³⁵ are classic mediators and have been widely used in wearable sensing systems, but their acute toxicity limits their continued usage and

underscores the need for a biocompatible encapsulation layer. Researchers are also exploring other materials, such as carbon-based nanomaterials,³⁶ metal nanoparticles and conductive polymers,³⁷ to mediate electron transfer in amperometric sensors.

Lastly, a biocompatible encapsulation layer is necessary for most wearable sensors. This encapsulation layer not only provides a skin-friendly interface, in theory, but also allows immobilized bio-receptors to maintain their bioactivity after long-term storage. Chitosan, glutaraldehyde and Nafion have been previously used as encapsulants, although the skin compatibility and cytotoxicity of these encapsulation approaches (particularly glutaraldehyde and Nafion) has yet to be established.

Future Needs and Prospects

Although remarkable advances in device form factor, flexibility and integrated circuit design have recently been achieved, notable hurdles, such as long-term biosafety, shelf life, reusability or wear resistance and longitudinal performance, remain unaddressed. Here, we highlight four areas of opportunity for future study that we believe will powerfully contribute to the accuracy, advancement, commercialization and adoption of wearable sensing systems.

Recognition layer.—Enzymatic recognition elements are widely used for sensing metabolites in sweat. Other recognition elements, such as bio-affinity immunoassays, that exhibit extremely strong binding between a target analyte and its bioreceptor may not be suitable for continuous on-body monitoring due to their inherently irreversible nature. Although enzymes provide high sensitivity and selectivity, they can be easily degraded by surrounding environmental factors, such as temperature, pH and humidity (too much or too little humidity), and fouled or compromised when diluted with nonspecific oily residues, makeup and microbiota present on human skin.¹⁹ Selected encapsulation techniques and polymer-enzyme composites have been shown to extend the shelf-life of enzyme-functionalized working electrodes;³⁸ however, this remains an area that requires ongoing attention and improvement.

Artificial receptors, such as biomimetic or molecularly-imprinted polymers (MIPs) are intriguing replacements for enzymatic

Table II. Categories of reported electrochemical devices.

Device Type	Mechanism	Application	References
Amperometric ^{a)}	Measures currents resulting from the oxidation or reduction of an electroactive analyte at a fixed potential	Detecting redox-active metabolites involved in enzymatic catalysis	13, 19, 20–22
Potentiometric ^{b)}	Measures the electrical potential created between a reference electrode and the working electrode through an ion/charge-selective membrane on the working electrode at close-to-zero current conditions	Detecting ions and charged molecules permeating through membranes	23–25, 22, 26
Impedimetric ^{c)}	Measures a resistance or capacitance change in a device across a finite range of applied bias frequencies following a biorecognition event	Label-free detection of proteins	27–29, 30

a) A three electrode cell is commonly used to avoid a voltage drop on the working electrode induced by counter electrode polarization. b) A liquid junction is always required by conventional potentiometric sensors, as it stabilizes the potential readout between two electrodes. But in terms of wearable applications, solution leakage may happen and cause skin irritation. This problem can be solved by fabricating electrodes coated with a KCl-saturated insulator layer or NaCl-saturated poly(vinylbutyral)(PVB) layer. c) Impedimetric sensing is usually achieved using a well-ordered self-assembled monolayer (SAM) created in between the recognition and transducer layers. The local environment of the SAM changes in the presence of different analytes, leading to a specific phase change.

recognition elements, particularly in wearable systems.³⁹ Metal-organic frameworks and nanomaterials have also been proposed.⁴⁰ In particular, MIPs are gaining recognition as a versatile tool for preparing synthetic polymers with tailor-made recognition sites and/or suitable ligand environments for specific analytes.⁴¹ Alberto et al. introduced a tailor-made polymeric membrane that facilitated the stable and selective molecular recognition of cortisol, a human stress hormone.⁹ Although these artificial receptors can be cheaper and more resistant to aging-induced degradation as compared to natural enzyme analogs, some limitations still need to be considered. Because their catalytic activity and analyte affinities are not as good as their enzymatic counterparts, electrodes functionalized with artificial receptors require more time to read out the data in real-time. Also, globally weaker selectivities may lead to false positives originating from nonspecific binding events.

Another problem of enzymatic recognition elements is the efficiency of signal transduction. Three common methods to transduce an enzymatic reaction into an electrical signal are used. The first is to sense a product or byproduct of the reaction.⁴² Famously, for example, oxygen serves as the final electron acceptor during glucose sensing and is reduced to peroxide by glucose oxidase (GOx). In this case, many working electrodes are built to sense the peroxide final product of the glucose sensing reaction and thus contain a combination of GOx along with horseradish peroxidase (HRP) or a Prussian Blue (PB) electrocatalyst, both of which sense peroxide.³⁹ However, this method of signal transduction requires complicated active layer formulations that may complicate the electrode fabrication process. Another method is to measure electrons shuttled to redox mediators. In this case, electrons from the target analyte are first transferred to the enzyme binding partner and then quickly transferred to the mediator, which leads to a change in the observed current that is proportional to the concentration of the target analyte. Since reactive sites can be buried deep within an enzyme, mediators can increase the total contact surface area and enhance electron transfer rates. Similar to the first instance, molecular electrocatalysts and metal/carbon nanomaterials are often employed as mediators but these compounds frequently lead to electrode decay by freely diffusing into the recognition layer instead of the conductive base. Moreover, many common mediators are highly cytotoxic and, therefore, can only be incorporated into wearable systems if thick encapsulation layers are used. The most efficient but the most difficult third method is to directly detect electron transfer from the enzyme active site to the electrode surface upon analyte binding. Highly-structured “template” layers can be used to immobilize enzymes close to the electrode surface and, thus, facilitate electron transfer. In Wang’s research,⁴ a mesoporous silicate foam (MCF) with pore diameters of 20 nm was designed and synthesized to immobilize Hb and GOx, realizing direct electron transfer from these enzymes to the underlying working electrode surface upon analyte binding. However, this strategy cannot be easily translated to flexible and wearable systems. Bioengineering strategies to realize artificial enzyme derivatives with exposed active sites have also been explored to create optimal recognition elements for electrochemical sensors.⁴³

In summary, enzymes are weak candidates for reusable sensors, even though they provide enviable chemo-specificity. Finding artificial substitutes that are cheaper and produced in bulk is desired, particularly for long-term health monitoring. Since skin provides the perfect large-area platform for distributed sensing networks, one possible strategy is to sacrifice chemo-specificity and use multiplexed sensors along with pattern recognition algorithms⁴⁴ to achieve signal accuracy in wearable systems.

Signal accuracy: false positives and false negatives.—Signal accuracy is compromised by surface fouling effects, which represent a major challenge to the continuous operation of wearable biosensors. Wearable biosensors can also be exposed to fluctuating conditions during prolonged outdoor activity that may affect the stability or activity of fragile bio-receptors. Achieving wearable

biosensors with long-term operational stability and signal accuracy requires a combination of proper receptor immobilization, protective (or target-selective) membranes, and storage conditions.

To ensure the reliability of the response during prolonged operation, robust antifouling surface protection is needed, along with active calibration mechanisms (multimodal, multianalyte sensing and drift correction). Biofouling is driven by the accumulation of interferent analytes on the sensor surface through nonspecific binding. Such rapid adsorption impedes diffusion and/or binding of the target analyte, leading to a gradual decrease of the sensing signal over time and eventual evolution of false negatives.

These issues can be partially addressed using microfluidic sampling systems and optimizing surface coating techniques.⁴⁵ Microfluidic systems, featuring multiple reaction chambers separated by using various valving techniques, can track time-dependent changes in analyte concentration in sweat. Also, calibrated analytes in different channels can collaboratively reveal sweat dynamics, as defined by instantaneous sweat rate and total sweat loss.²⁸ For example, Kim et al. used⁴⁶ patterned hydrophobic microchannels as valves to divert sweat toward different reaction zones, while sweat-absorbent swellable polymers were used at the inlet of each zone to block sweat flow and further isolate the reaction site.

Accurate on-body measurements also require active removal of old fluids. Washing away analytes after each sensing event will significantly enhance the long-term performance of wearable electrochemical sensors and reduce the occurrence of false positives due to incomplete target clearance after a singular recognition event. In this case, hydrogel coatings or shells are promising due to their viscoelastic properties, which respond to various external stimuli like electrical field and pH, and tunable affinities for different biological components.⁴⁷ For example, an ionic hydrogel can reversibly swell and de-swell relative to an aqueous condition and therefore enable excess analytes to flow away. Hydrogels with a high dielectric constant can undergo voltage-induced, reversible deformations that can mechanically effect interferent unbinding.¹

Wear resistance and reusability.—Temporary tattoo sensors, tiny and adhesive, can be easily fixed on human skin and have presented excellent efficiency to date. However, with respect to long-term usage, some limitations should be carefully considered. Tattoo sensors, or other tight-fitting sensors, usually require skin preparation in advance, such as hair shaving and alcohol cleaning of the contact area, which largely excludes them from widespread use and everyday wear. The surface chemistry/biochemistry of the wearer’s skin may also influence sensing performance. For example, the adhesive glue used to affix the sensor to skin may dry out and cause the device to shift or fall-off.⁴⁸ Dissolution of the active layers may also occur when the sensor is exposed to large quantities of sweat, which will lead to signal degradation and evolution of false negatives, in addition to skin irritation if any of the active layer components are not biocompatible. Lastly, considering the metastable adhesive interaction between the tattoo and the wearer’s skin, the ability of such skin-mounted devices to withstand continuous body stretching, fracturing and slipping is not assured.¹

Fabric-based and/or garment-integrated sensors.—Conceptually, textiles and garments, which we wear in daily life, provide a pervasive platform for distributed sensing networks while also exhibiting excellent mechanical properties and longevity. With the aid of efficient knitting and weaving methodologies, functional fibers can be easily integrated into textiles and garments. Moreover, the ordered structure of textiles is naturally suitable for an electrochemical sensor composed of three adjacent electrodes.⁴⁹

Although various iterations of fiber- and fabric-based colorimetric sensors are known,^{7,50} fibers and fabrics have not been widely investigated as platforms for electrochemical sensors. A long-standing challenge is creating a high-performing working electrode directly on the surface of textiles that also displays stability against the harsh mechanical and chemical stresses to which textiles and

garments are regularly subject. Existing fabrication methods (mentioned previously) work best with a flat, uniform substrate, thus largely excluding fibers and off-the-shelf textiles. Furthermore, the loose structure of textiles can easily deform printed conductive layers and lead to delamination.

Recently, selected researchers have demonstrated the use of specialized conductive fibers, such as carbon fibers,⁵¹ stainless steel yarns,²⁴ metallic fibers,⁵² and silver-coated nylon threads⁵³ as both platforms for fiber-based working electrodes and as interconnects for distributed sensing systems. Wang et al. created fiber-based working electrodes by electrodeposition of active materials onto carbon fibers and integrating these fibers into a sensing array.²⁵ In Zhao's work, a thin gold fiber made by dry-spinning gold nanowires was used for glucose sensing.⁵⁴

Depositing conductive polymers on commercially-available garments and textiles is an alternative way. However, caution should be exercised when using solution-based deposition processes to make polymer coatings (for example, electrochemical deposition, in situ solution polymerization and dipcoating) because many off-the-shelf fibers and fabrics textiles may get impregnated with the evolving polymer and/or mechanically deformed by the solvents used during the synthesis process.³⁶ Thanks to nascent, creative polymer synthesis approaches, these problems can be overcome—conductive polymers can be deposited on arbitrary textiles via reactive vapor deposition, which curtails solvent use and enables rugged and conformal polymer coatings that are remarkably laundering-stable and resistant against fouling with continued wear. Andrew et al.²⁷ used reactive vapor deposition to create garment-embedded fabric sensors to detect various body motions.

Summary

The promise of real-time, personalized health care has driven exciting and multidisciplinary work on fabricating various forms of wearable and/or skin-mounted electronic sensors. In this short review, we highlight major advances in creating wearable electrochemical sensors for longitudinal health monitoring. Although rapid progress has been made in the past few years to realize wearable electrochemical sensors with high sensitivity and chemo-selectivity, limitations in long-term performance, shelf life and reusability are waiting to be overcome. Here, four attractive “targets of opportunity” are proposed, ranging from active layer development to novel device platforms and systems-level approaches, to address these challenges. We expect that collaborative endeavors from researchers with diverse expertise will contribute significantly to driving this area forward.

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