

# Wet Interface Engineering for At-rest Sweat Analysis

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Biosensors capable of monitoring physiological conditions from compounds collected noninvasively from external bodily fluids have been actively developed to realize personalized predictive medicine. Sweat is one of the most attractive bodily fluids for analysis of physiological conditions because of its easy access to the human skin surface and as a potential source of blood-derived biomarkers. However, the repetitive sampling of at-rest sweat required for biosensing is one of the greatest challenges in achieving the continuous monitoring of human health. In this paper, we summarize the recent development of a new type of biosensor that effectively and repeatedly collects and detects at-rest sweat components in a safe and easy manner by employing a hydrogel touchpad-based wet interface between the device and skin. In the future, the hydrogel touchpad-based sweat biosensor will allow individuals to track changes in sweat composition to realize daily self-health management.

## 1. Introduction

The development of chemical sensing devices for self-health management that can monitor individual health conditions in real time and provide personalized feedback to induce behavioral changes without clinical visits is a key issue because such devices are expected to support the realization of personalized predictive medicine. Miniaturized biosensors, which are analytical devices that utilize biometric elements connected to various signal transducers, have been intensively studied as simple, user-friendly sensing devices for point-of-care-testing applications, especially for self-glycemic management in diabetic patients.<sup>(1)</sup> Recently, the sensitivity of biosensors has improved dramatically, making it possible to detect trace components in external bodily fluids and gases, such as saliva, tears, sweat, skin gas, and breath. Comprehensive analysis of these external body fluids and gases has revealed the presence of possible blood-derived biomarkers.<sup>(2,3)</sup> More importantly, these fluids and gases can be easily withdrawn from the body without causing any physical pain, which makes it possible to perform routine self-management of physiological conditions based on the compositions of these fluids and gases without painful blood sampling. In addition, advances in biosensor materials and processing have made it possible to manufacture ultrathin, light, and soft miniaturized biosensors that can be worn on the human skin<sup>(2,4)</sup> as well as in the oral cavity<sup>(5)</sup> and ears.<sup>(6)</sup> These advances allow

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both patients and healthy individuals to use personalized portable and wearable biosensors for noninvasive daily self-health management.

Among the external biofluids and gases, sweat is one of the most attractive options for analysis of physiological conditions because it can be easily collected from the skin. Although human sweat is primarily composed of water (99%), it is believed that the remaining 1% of trace components contain biomarkers that reflect the current physiological state, as summarized in previous review articles.<sup>(7,8)</sup> Therefore, the integration of several biosensors into a single device is necessary for reliable sweat-based diagnosis.

However, one of the biggest challenges in sweat analysis is how to collect the large amounts of sweat required for on-demand biosensing. To overcome this issue, we have recently developed a new type of sweat biosensor that can easily extract sweat components at any time by employing a wet interface between the sensor and human skin.<sup>(9–12)</sup> We named it the “hydrogel touchpad-based sweat biosensor” (Fig. 1). In this paper, we describe the operational principle of the sensor and summarize the recent progress and limitations of this type of sensor to discuss future prospects for at-rest sweat-based noninvasive diagnosis.

## 2. Wet Interface for Extraction of At-rest Human Sweat Components

Sweat forms on the skin in many situations, such as after exercise or bathing, or when wearing closed gloves. Visible sweat can be easily collected using filter paper, cotton, gauze, or towels; however, passive sampling of sweat that occurs under normal conditions cannot be used for daily diagnosis. Another strategy for inducing sweat is the iontophoresis-assisted administration of cholinergic agonists such as pilocarpine, which actively induce sweat.<sup>(13)</sup> Iontophoresis is a technique used to enhance the transdermal introduction of chemical compounds into the body by applying a low-density direct current through the skin surface. Sweat biosensors integrated with iontophoresis enable on-demand monitoring of sweat component concentrations. However, repeated daily drug administration may be uncomfortable for users.

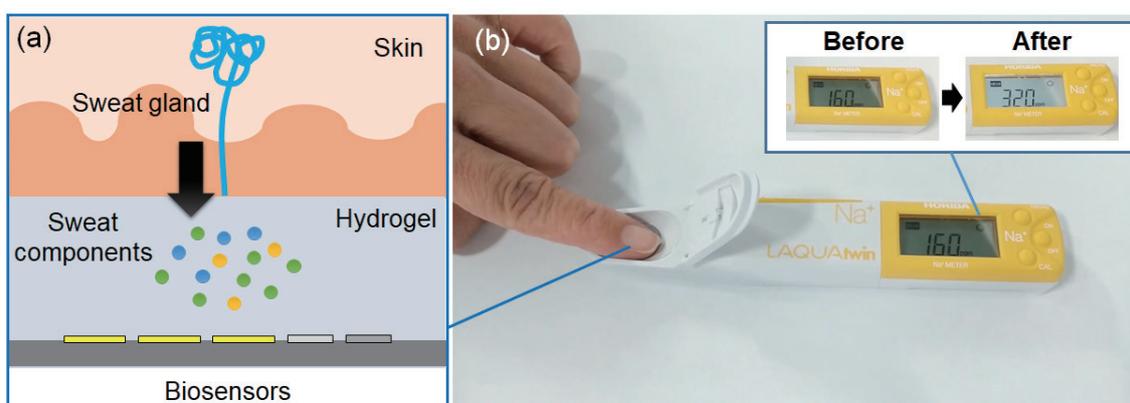


Fig. 1. (Color online) (a) Cross section of wet interface between human skin and hydrogel touchpad-based sweat biosensor. (b) Photograph of a touchpad-based sweat Na<sup>+</sup> sensor prototype.

A simple and safe technique for repeatedly collecting at-rest sweat components has been established in the field of sweat omics. Researchers have extracted sweat components by exposing human skin to a water-based extraction solution that caused sweat components to diffuse out of the sweat gland.<sup>(14–19)</sup> They performed comprehensive sweat omics analysis using large-scale analytical systems such as liquid chromatography and mass spectrometry systems. However, if the osmotic pressure of the extraction solution is lower than that of the epidermal layer of the skin, the stratum corneum may swell and the sweat glands may close.<sup>(20)</sup> To avoid this phenomenon, phosphate-buffered saline (PBS) is a potential extraction solution.<sup>(21–26)</sup>

### 3. Integration of Hydrogel-based Wet Interface into Electrochemical Biosensors

On the basis of these reports, we developed a novel hydrogel touchpad-based sweat sensor for the extraction and detection of at-rest sweat components [Figs. 1 and 2(a)].<sup>(9)</sup> The sensor consisted of a lactate oxidase (LOx)-based L-lactate sensor and a Ag/AgCl reference electrode completely covered with an agarose hydrogel containing PBS solution. Sweat L-lactate was extracted from sweat glands in human skin into the hydrogel by simply touching the hydrogel. This was followed by *in situ* electrochemical detection using the L-lactate biosensor. After the subject placed their finger on the hydrogel surface, the sensor signal increased and then remained steady. To the best of our knowledge, this is the first report showing a proof of concept of a hydrogel touchpad-based extraction and sensing device for at-rest sweat components. Recently, a planar liquid-junction reference electrode has been integrated into this sensor as a reference electrode, which improves the stability of the reference electrode potential even when the Cl<sup>-</sup> ion concentration changes when extracting sweat Cl<sup>-</sup> ions into the gel.<sup>(11)</sup>

Hydrogel touchpad-based sweat sensors have evolved over the past few years, as summarized in Table 1. Lin *et al.* developed a thin agarose gel-modified electrochemical sensor for sweat L-lactate sensing.<sup>(27)</sup> The structure of the sensor electrode was gold/multiwalled carbon nanotube/platinum nanoparticle-modified electrode with lactate oxidase immobilized on its surface. The sensor showed a linear response in the concentration range from 0 to 4 mM with a sensitivity of  $1.88 \pm 0.24 \mu\text{A}/(\text{mM cm}^2)$ . The limit of detection was  $0.12 \pm 0.02 \text{ mM}$ . In addition, they established a wireless sensing network on a cloud system to collect and analyze user data.

Table 1  
Summary of recently developed hydrogel-touchpad-based sweat biosensors.

Biomarker	Detection method	Reference
L-lactate	Electrochemical method	9
L-lactate	Electrochemical method	27
L-lactate	Electrochemical method (Self-powered biosensor)	28
D-glucose	Electrochemical method	29
D-glucose	Electrochemical method	30
Cl <sup>-</sup>	Electrochemical method	11
Cortisol	Electrochemical method	31
L-dopa	Electrochemical method	32
pH, D-glucose, Cl <sup>-</sup> , Ca <sup>2+</sup>	Colorimetry	33
pH, Cl <sup>-</sup> , D-glucose, L-dopa, perspiration rate	Electrochemical method	34

Yin *et al.* developed a self-powered hydrogel touchpad-based sweat lactate biosensor using an enzyme-based biofuel cell (BFC).<sup>(28)</sup> The system consisted of a LOx anode and a Pt cathode completely covered with a porous polyvinyl alcohol (PVA) hydrogel. When subjects placed their fingers on the PVA hydrogel, sweat L-lactate was extracted into the gel as fuel for the enzyme-based BFC system, which generated an amount of electrochemical power depending on L-lactate concentration. This operational principle implies that the generated electrochemical power represents the extracted L-lactate. The generated power was confirmed by the color change in the poly(3,4-ethylenedioxy)thiophene:poly(styrene sulfonate) (PEDOT:PSS)-based electrochromic display, which was powered by the touch-based BFC. Sempionatto *et al.* developed a glucose oxidase (GOx)-based electrochemical sweat glucose sensor covered with a thin porous PVA hydrogel [Fig. 2(b)].<sup>(29)</sup> Sweat glucose is a promising target for the noninvasive and indirect estimation of blood glucose levels. They quantified the correlation between blood and sweat glucose levels and established personalized parameters to accurately estimate the blood glucose levels from signals from the touchpad-based sweat glucose sensor. These parameters were used to monitor sweat glucose levels throughout the day to verify the accuracy of the touchpad-based sweat glucose sensor and validate its applicability for daily diagnosis. Lin *et al.* developed a highly sensitive and stable GOx-based sweat glucose sensor utilizing a Prussian blue (PB)-doped PEDOT nanocomposite [Fig. 2(c)].<sup>(30)</sup> PEDOT plays an important role in the stable immobilization of PB particles on the electrode with reducing the interfacial impedance. This sensor showed high sensitivity to glucose in the concentration range of 6.25  $\mu\text{M}$  to 0.8 mM with a limit of detection of 4  $\mu\text{M}$ . Sensor electrodes covered with thin agarose

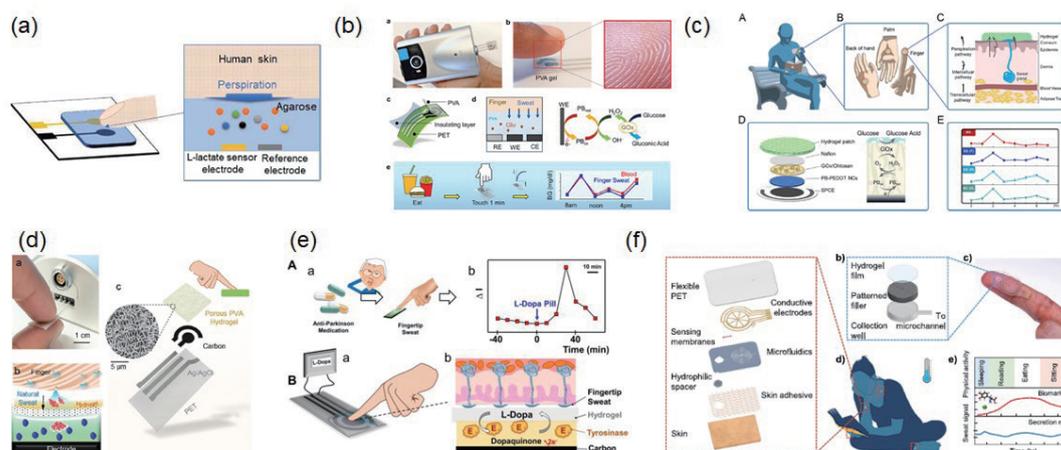


Fig. 2. (Color online) Examples of recently developed hydrogel touchpad-based sweat biosensors. (a) Agarose hydrogel-based electrochemical sweat lactate sensor (reproduced with permission from Ref. 9, <http://creativecommons.org/licenses/by/4.0/>). (b) GOx-based electrochemical glucose sensor covered with a thin porous PVA hydrogel (reprinted with permission from Ref. 29, Copyright (2021) American Chemical Society). (c) GOx-based sweat glucose sensor utilizing a PB-doped PEDOT nanocomposite (reproduced with permission from Ref. 30, <https://creativecommons.org/licenses/by-nc-nd/4.0/>). (d) MIP-based electrochemical sweat cortisol sensor (reproduced with permission from Ref. 31). (e) Tyrosinase-based electrochemical sweat L-dopa sensor (reproduced with permission from Ref. 32). (f) Hydrogel touchpad-integrated wearable microfluidic device for sweat component detection (reproduced with permission from Ref. 34, <http://creativecommons.org/licenses/by/4.0/>).

hydrogel in pure water were attached to various parts of a subject (fingers, palms, backs of hands) to monitor local sweat glucose throughout the day and compare changes in blood glucose levels. Tang *et al.* developed a molecularly imprinted polymer (MIP)-based electrochemical sweat cortisol sensor [Fig. 2(d)].<sup>(31)</sup> The MIP on the electrode contained PB as a redox signal generator. The signal decreased with increasing cortisol concentration. The sensitivities in PBS and artificial sweat were 38.8 nA/log [nM] (1 to 10<sup>5</sup> nM) and 60.31 nA/[nM] (10 to 10<sup>3</sup> nM), respectively, with detection limits of 0.9 in PBS and 0.2 nM in artificial sweat. These sensitivities covered the concentration range of extractable sweat cortisol levels and demonstrated temporal monitoring of changes in sweat cortisol concentration during the day. Moon *et al.* tracked sweat L-dopa levels following a single dose of an L-dopa/carbidopa oral tablet [Fig. 2(e)].<sup>(32)</sup> Tyrosinase immobilized on the carbon electrode oxidized sweat L-dopa to dopaquinone, which was reduced at the electrode to produce an amperometric reduction current related to the L-dopa concentration. The sensor showed a linear response in the concentration range of 1–30  $\mu$ M, and the estimated limit of detection was 300 nM. A touchpad-based electrochemical L-dopa sensor was fabricated by covering the electrodes with thin porous PVA, and L-dopa was intermittently monitored in the extracted sweat by comparing blood L-dopa levels. The authors emphasized that the dynamic profiling of sweat L-dopa can help establish guidelines for the personalized treatment of patients with Parkinson's disease. Recently, Wang *et al.* have reported a colorimetric touch-based sensor.<sup>(33)</sup> The hydrogel touchpad consisted of colorimetric probes for pH, glucose, Cl<sup>-</sup>, and Ca<sup>2+</sup> arrayed on the same adhesive hydrogel substrate. This adhesive hydrogel film easily adhered to human skin and exhibited *in situ* color changes depending on the concentration of the extracted sweat components. These colors were captured with a camera integrated into a smartphone, and the RGB digital signal was calculated from the captured images.

These hydrogel-based wet interfaces can be easily integrated into commercially available chemical sensors to demonstrate reliable sweat sensing, as shown in Fig. 1(b). The active area of a sodium ion sensor purchased from Horiba (LAQUAtwin Na-11) was covered with filter paper soaked in PBS. The PBS originally contained 130 ppm of sodium ions, and after contact, the detected value increased to 320 ppm, which means that 190 ppm of sodium ions were extracted into the PBS. We believe that this strategy is a straightforward way to accelerate the application of hydrogel touchpad-based sweat sensors.

#### 4. Limitation of the Hydrogel Touchpad-based Sweat Sensors

Conventional touchpad-based sweat sensors have difficulties in normalizing detected signals because it is challenging to quantify the sweat volume extracted into the hydrogel. Therefore, it has not been possible to determine whether changes in the concentration of sweat compounds or changes in the extracted sweat volume were responsible for changes in sensor signals. Quantitative evaluation of changes in the detected signal based on the extracted sweat volume is important for reliable diagnosis. Nyein *et al.* provided an important solution to this problem by combining a hydrogel touchpad with the inlet of a polydimethylsiloxane-based microfluidic channel [Fig. 2(f)].<sup>(34)</sup> The at-rest sweat extracted into the hydrogel touchpad composed of PVA/agarose in glycerol was directly introduced into the microchannel, and the extracted sweat

volume was calculated from the microchannel dimensions. With this device, the perspiration rate of the at-rest sweat volume in the hydrogel touchpad was found to be  $0.1\text{--}1\ \mu\text{L min}^{-1}\ \text{cm}^{-2}$  at a human finger. However, if the flow velocity of at-rest sweat entering the channel is very low, the concentration of sweat components previously filled in the channel may be averaged when mixed with the newly introduced sweat. Thus, there still appears to be a problem in measuring changes in sweat components in real time.

Another issue is the dehydration of the hydrogel touchpad during use, which prevents long-term continuous and quantitative monitoring of sweat components extracted into the hydrogel. Nonvolatile gels have been extensively studied in the field of electrolyte-gated transistors and soft conductors. Employing gels containing nonvolatile solutions such as ionic liquids,<sup>(35)</sup> glycerol,<sup>(36)</sup> and humectants (highly concentrated NaCl and LiCl)<sup>(37,38)</sup> is a potential solution to this problem. However, most nonvolatile solutions are cytotoxic or highly viscous. In particular, highly viscous solutions are not compatible with electrochemical measurements owing to their low ion conductances. The development of nonvolatile, biocompatible, ion-conductive, and sweat-extractable gels is still a challenging issue for the ideal continuous extraction of at-rest sweat while monitoring sweat volume.

## 5. Conclusions and Future Perspectives

In this paper, we describe recent progress in hydrogel-based wet interface engineering for noninvasive sweat collection and analysis at rest. Hydrogel touchpad-based sweat sensors are now being developed to realize the quantitative detection of sweat components by considering the extracted sweat volume and dehydration of the gel. This type of sensor is simple, safe, and user-friendly, and is suitable for daily use not only for healthy people, but also for clinical patients and elderly individuals. On the other hand, medical evidence regarding the correlation between diseases and sweat components is still insufficient. This simple and safe sweat extraction technique has significant potential for accelerating sweat omics analyses. We believe that parallel progress in both sensor development and omics analysis will realize reliable evidence-based medical diagnosis using at-rest sweat in the future.

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## Ethical Approval

All the experimental procedures performed in Fig. 1 involving human participants were in accordance with the standards of Ethics Committee of Faculty of Medicine, Yamagata University (Approval No. R3-39).

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