Supporting Information

Photosensitive-Stamp-Inspired Scalable Fabrication Strategy of Wearable Sensing Arrays for Noninvasive Real-Time Sweat Analysis

Junxing Hao,† Zeqiang Zhu,§ Chengguo Hu,‡ and Zhihong Liu,†;,*

† College of Chemistry and Chemical Engineering, Hubei University, 430062 Wuhan, China.

‡ College of Chemistry and Molecular Sciences, Wuhan University, 430072 Wuhan, China.

§ School of Mechanical Science and Engineering, Huazhong University of Science and Technology, 430074 Wuhan, China.

Email for Z.L.: zhhliu@whu.edu.cn
Table of Contents

**EXPERIMENTAL SECTION**: Preparation of glucose and lactate enzyme-based sensors; Preparation of Na\(^+\) and K\(^+\) ions-selective sensors; Signal acquisition and processing circuit system design; Power delivery to the intelligent wearable sweat sensor; Design of customized mobile phone application (app); Ex-situ sweat analysis; In-situ sweat analysis

**Figure S1.** Fabrication process of the patterned photosensitive stamp (PS)
**Figure S2.** Photos of vacuum filtration devices
**Figure S3.** Transfer printing process of SWCNT-based flexible electrode arrays
**Figure S4.** The diagram of signal acquisition and processing circuit system
**Figure S5.** The calibration curves of flexible circuit output
**Figure S6.** Power delivery diagram of the intelligent wearable sweat sensor
**Figure S7.** The customized mobile phone app for data analysis
**Figure S8.** The photos and SEM images of PS
**Figure S9.** The elaborated and complicated pattern of PS
**Figure S10.** Photos of PS-inspired pattern
**Figure S11.** SEM and TEM images of SWCNTs and SWCNTs-Pt
**Figure S12.** Raman spectra of SWCNTs-Pt-PB and SWCNTs-Pt-PEDOT
**Figure S13.** XPS spectra of SWCNTs-Pt, SWCNTs-Pt-PB, and SWCNTs-Pt-PEDOT
**Figure S14.** CVs of SWCNTs, SWCNTs-Pt, SWCNTs-Pt-PEDOT, SWCNTs-Pt-PB
**Figure S15.** CVs of glucose and lactate sensors by SWCNTs, SWCNTs-Pt, and SWCNTs-Pt-PB
**Figure S16.** The potential stability tests
**Figure S17.** The selectivity tests
**Figure S18.** The reproducibility tests
**Figure S19.** The storage life tests

**Table S1.** The cost of a PS-VFTP based flexible electrode array
**Table S2.** The comparison of sensitivity of recently reported wearable sweat sensors

REFERENCES
EXPERIMENTAL SECTION

Preparation of glucose and lactate enzyme-based sensors.

A homogeneous 0.5 % Chi solution was first obtained by means of dissolving Chi in 2 % HAc and magnetic stirring for 1 h, and then the Chi solution was mixed with SWCNTs (2 mg mL\(^{-1}\)) by ultrasonic processing for 30 min to produce a viscous solution of Chi/SWCNTs. For the glucose sensors, the Chi/SWCNTs solution was mixed adequately with GOx solution (5 mg mL\(^{-1}\)) in a volume ratio of 1:1 (GOx/Chi/SWCNTs). A thinner Prussian blue (PB) layer was deposited on the SWCNTs-Pt electrode by applying a potential of 0.4 V (versus Ag/AgCl) for 100 s in a freshly prepared solution containing 2.5 mM FeCl\(_3\), 2.5 mM K\(_3\)[Fe(CN)\(_6\)], 0.1 M KCl, and 0.1 M HCl (A thinner PB layer can offer superior sensitivity for the detection of low level glucose in sweat). The glucose sensor was attained by drop-casting 6 μL the mixture of GOx/Chi/SWCNTs on the SWCNTs-Pt-PB electrode. To prepare the lactate sensors, A thicker PB layer was deposited on the SWCNTs-Pt electrode by applying a potential of 0.4 V (versus Ag/AgCl) for 480 s in a freshly arranged solution containing 2.5 mM FeCl\(_3\), 2.5 mM K\(_3\)[Fe(CN)\(_6\)], 0.1 M KCl, and 0.1 M HCl (A thicker PB layer can provide a wider detection linearity for the measurement of lactate in sweat). Whereafter, 3 μL of the Chi/SWCNTs solution was drop-casting on the SWCNTs-Pt-PB electrode and drying at room temperature. Finally, the electrode was successively modified with 6 μL of the LOx solusion (2 mg mL\(^{-1}\)) and 3 μL of the Chi/SWCNTs solution. These enzyme-based sensors were arranged to dry at 4°C without light overnight. Besides, the stock solutions were kept at 4°C when not in use. It should be
further noted that the two-electrode system is a universal strategy for highly sensitive electrochemical sensor. As regards amperometric glucose and lactate sensors, the Ag/AgCl electrode is treated as both reference and counter electrodes to simplify circuit design and benefit system integration. The output currents of the glucose and lactate sensors are transformed to the readable potential via a transimpedance amplifier. Considering the low level of glucose in sweat, the as-designed sensors are 3 mm in diameter to acquire a high sensitivity (the larger electrode area offering larger detection sensitivity).

**Preparation of Na\(^+\) and K\(^+\) ions-selective sensors.**

The conducting film of poly(3,4-ethylenedioxythiophene) (PEDOT) was selected as an excellent ion-electron transduction medium to diminish the potential drift of the ion selective electrodes (ISEs) and deposited on the working electrodes (SWCNTs-Pt) by galvanostatic electro-polymerization process with a solution containing 0.01 M EDOT and 0.1 M NaPSS (versus Ag/AgCl) at current density of 0.2 mA cm\(^{-2}\) and polymerization charges of 10 mC (SWCNTs-Pt-PEDOT). The Na\(^+\) selective membrane mixture comprised of Na\(^+\) ionophore, Na-TFPB, PVC, and DOS with 1 %, 0.55 %, 33%, and 65.45% (weight by weight, w/w), respectively. 200 mg of the membrane mixture was fully dissolved in 1.32 mL of THF. Furthermore, the K\(^+\) selective membrane mixture consisted of K\(^+\) ionophore, NaTPB, PVC, and DOS with 2 %, 0.5 %, 32.8 %, and 64.7 % (w/w), respectively. 200 mg of the membrane mixture was totally dissolved in 0.7 mL of CYC. The mixture of the PVB-based reference electrode was synthesized by dissolving 79.1 mg of PVB and 50 mg of NaCl in 1 mL methanol. Moreover, 2 mg
of F127 and 0.2 mg of MWCNTs were added into the PVB-based reference solution to further diminish the potential drift. These ion selective membrane and reference solutions were sealed and stored at 4 °C before use. Eventually, Na⁺ and K⁺ ISEs were fabricated via drop-casting 6 μL of the Na⁺ selective membrane mixture and 4 μL of the K⁺ selective membrane mixture on the SWCNTs-Pt-PEDOT electrodes. The shared reference electrode for the Na⁺ and K⁺ ISEs was improved by drop-casting 10 μL of PVB-based reference solution on the Ag/AgCl electrode (PVB-Ag/AgCl). The modified electrodes were positioned at constant temperature and humidity conditions to dry overnight. In order to further minimize the potential drift of the Na⁺ and K⁺ ISEs, these ISEs were severally incubated with 0.1 M NaCl and 0.01 M KCl for 1 h before detection.

**Signal acquisition and processing circuit system design.**

The circuit system of signal acquisition and processing was designed based on the characteristics of weak electrochemical signals outputs by glucose, lactate, Na⁺, and K⁺ sensors. The sensor can simultaneously monitor four target analytes, so it needs four road signal processing circuit to process, then collects and reads the corresponding voltage value via microcontroller Analog-to-digital Converter (ADC) module. Finally, the Bluetooth module transmits the data to the Arduino device, so that the users can read health-related molecular level information. Based on the above ideas, the STM32F103C8T6 32-bit microcontroller (STMicroelectronics) was selected as the control center of circuit system. As is well-known, STM32 has the characteristics of advanced architecture, high performance, low voltage, low power consumption,
abundant peripherals, and easy to use. Herein, the ADC function module of STM32 was mainly employed. The STM32F103 series have three ADCs with a precision of 12 bits, and each ADC has up to 16 external channels. Although the sensor in this project has four target analytes, the four external channels of two ADCs can be utilized for alternate measurement. The Bluetooth module adopted the CC2541 chip with 256 KB space from Ti company in the United States, and followed the specification of V4.0BLE Bluetooth. In addition, this module has a serial port, and the advantages of low cost, small size, low power consumption, and high sensitivity in receiving and sending. After the main modules were selected, the sensor acquisition circuit will be described in detail, as shown in Figure S4. To construct a high-sensitivity sweat sensor, it was necessary to amplify the weak current generated by glucose and lactate in sweat, and perform filtering and other methods to process this response signals. Firstly, the current signals needed to be converted into a voltage signal using an inverted proportional operation circuit (IPOC) and amplified by half a million times, so that the input voltage to Microcontroller Unit (MCU) can be reached at a readable range. As the IPOC was applied, the voltage was negative at this time. This required the use of an IPOC again to convert the negative voltage into a positive voltage, and finally the clutter was filtered by a fourth-order low-pass filter circuit, and the remaining voltage signals were imported into the ADC module of MCU. As to Na⁺ and K⁺ sensors, the direct output voltages between ISEs and the reference electrode were the range of 0-3.3 V, so there was no need to further amplify the voltage signal, but it was essential to employ a differential subtraction circuit to convert the two input signals into one signal.
Whereafter, the clutter was filtered by a fourth-order low-pass filter circuit, and finally it was imported into ADC module to read the voltage values. As shown in Figure S5, all four signal-regulation channels indicate an outstanding linear response (correlation coefficient, $R^2 = 1.00$). In order to eliminate the non-ideal effects for instance voltage offset and obtain accurate signal readings, the precise digital linear relationship between the output and input was achieved to map the pristine input signal to analogue circuit readouts, thus permitting following signal calibration and processing at the software level. After the data was processed and averaged, the microcontroller was applied to transmit the data to Bluetooth module for wireless transmission.

**Power delivery to the intelligent wearable sweat sensor.**

The intelligent wearable sweat sensor was powered by a rechargeable lithium battery power supply with a nominal voltage of 5 V, the battery and circuit board interface using common USB on the market (Figure S6). According to power supply characteristics of the operational amplifier, it owns a -5 V voltage conversion module, which can convert 5 V to -5 V. The power supply voltage of STM32 microcontroller and Bluetooth module is 3.3 V, so it also owns a 3.3 V voltage conversion module.

**Design of customized mobile phone application (app).**

When the underlying data processing module was completed, the data needed to be transmitted to customized mobile phone app using the Bluetooth module. As shown in Figure S7, the app has made some improvements based on BluetoothLeGatt (Android) or LightBlue (IOS), and can read the concentration values of the corresponding parameters (glucose, lactate, Na⁺, and K⁺). A specialized app will be
designed for data analysis and storage as needed, and the health-related data will be presented to users with a more friendly and visualized human-computer interaction interface.

**Ex-situ sweat analysis.**

The ex-situ sweat analysis was implemented by detecting sweat specimens derived from the volunteers’ arm. Sweat specimens were collected every 5 min by scraping the sweat of arm with microtubes (0.3 mL each time, 5 times in total), and volunteers’ arm was wiped and cleaned with gauze after every sweat collection. The concentrations of glucose and lactate in volunteers’ sweat were measured using a high-performance liquid chromatography-mass spectrometry (HPLC-MS) and the levels of Na$^+$ and K$^+$ were detected on an inductively coupled plasma-optical emission spectrometer (ICP-OES). For the detailed experimental process, please refer to the national standards of China including GB/T 30986-2012, GB/T 23877-2009, and GB/T 40272-2021.

**In-situ sweat analysis.**

The noninvasive real-time sweat analysis of intelligent wearable sensors was performed in compliance with the protocol that was approved by the institutional review board at the Wuhan University. A healthy volunteer (female), ages 30, was recruited from the Hubei University. She gave the written informed consent before participation in this project. This project was that a volunteer wearing the intelligent wearable sweat sensor on her wiped and cleaned wrist, defined as “intelligent wristband”, allowing for continuous, real-time, and in-situ sweat analysis during Nintendo Switch fitness exercise (Just Dance). A bibulous thin sponge mat was
positioned between the skin and flexible electrode arrays during in-situ monitoring to absorb and keep adequate sweat, which contributed to acquiring the stable and reliable sensor readings, and also can avoid direct mechanical contact between flexible electrode arrays and skin. The sponge mat can absorb approximately 15 μL of sweat, which is enough to supply the stable and continuous sensor readings. During in-situ experiments, the freshly produced sweat will replenish sponge mat and wash away the old sweat. The volunteer was asked for continuous exercise about 35 min, during which sweat information was captured by the intelligent wristband, and the health-related data were transferred to a customized mobile phone app via signal acquisition and processing circuit system. In addition, to verify the accuracy of PS-VFTP based intelligent wearable sweat sensors, the in-situ monitoring results need to be compared with the ex-situ measurements by highly reliable golden standard approaches.
Figure S1. (A) Pattern, (B) the patterned tracing paper, (C) flash stamp machine, and (D) a photosensitive stamp after exposing with (B).
**Figure S2.** Photos of vacuum filtration devices for preparing SWCNTs-based flexible electrode arrays. (A) A porous titanium plate (diameter 0.5 cm, pore size 0.45 μm, Shijiazhuang Yida Filter Equipment Co.,) and a patterned PVDF filter membrane. (B) Separated devices. (C, D) Integrated devices (the inset of D is SWCNTs and diluted SWCNTs dispersion solutions).
Figure S3. (A) SWCNTs/PVDF arrays was covered with liquid PDMS. (B) PDMS/SWCNTs/PVDF after curing. (C) the PVDF film was peeled off from the side of SWCNTs/PDMS.
Figure S4. The circuit system of signal acquisition and processing for glucose, lactate, Na\(^+\), and K\(^+\). The VCC and VSS are positive and negative power sources, respectively. The LT1462CS8 is the chip part of integrated circuits.
Figure S5. The calibration curves of intelligent sensor output for glucose (A), lactate (B), Na\(^+\) (C), and K\(^+\) (D).
Figure S6. (A) Power delivery diagram of the intelligent wearable sweat sensor. (B) Photo of a small rechargeable lithium battery power supply. (C) The signal acquisition and processing circuit system connected to power supply (B).
Figure S7. The customized mobile phone app for data analysis. (A) The customized mobile phone app, (B) the page of the app during Bluetooth pairing, (C) the real-time data analysis of sweat analyte (glucose, lactate, Na\(^+\), and K\(^+\)) concentrations during exercise.
Figure S8. The photos and SEM images of PS before (A-D) and after (E-H) exposure.
Figure S9. (A-D) Patterned templates of school badge, butterfly, lines, and electronic circuits. (E-H) Corresponding photosensitive stamps after exposure.
Figure S10. Photos of PS-inspired pattern. (A) Patterned PS filled with PDMS. (B) PDMS/PVDF template. (C) SWCNTs/PVDF-based pattern. (D) SWCNTs/PDMS based flexible electrode arrays. (E, F) SWCNTs-based school badge and butterfly. (G) The resolution of different width lines. (H) SWCNTs-based electronic circuits.
Figure S11. (A, B) SEM and (C, D) TEM images of (A, C) SWCNTs and (B, D) SWCNTs-Pt.
Figure S12. (A, B) Raman spectra of SWCNTs-Pt-PB and SWCNTs-Pt-PEDOT.
Figure S13. (A, B) XPS spectra of the C 1s and Pt 4f states in SWCNTs-Pt. (C) XPS spectra of the Fe 2p states in SWCNTs-Pt-PB. (D) XPS spectra of the S 2p states in SWCNTs-Pt-PEDOT.
Figure S14. CVs of (A) SWCNTs, (B) SWCNTs-Pt, (C) SWCNTs-Pt-PEDOT, and (D) SWCNTs-Pt-PB in 0.2 M KCl at different scan rates (25, 50, 75, 100, 125, and 150 mV s\(^{-1}\)).
Figure S15. CVs of glucose (A) and lactate (B) sensors by SWCNTs (I), SWCNTs-Pt (II), and SWCNTs-Pt-PB (III) in 0.1 M phosphate buffers (pH 7.0). The scan rate is 50 mV s$^{-1}$. 
**Figure S16.** Potential stability of a customized PVB-based Ag/AgCl electrode, a customized Ag/AgCl electrode, and a commercial aqueous Ag/AgCl electrode in 4 mM K⁺ solutions.
**Figure S17.** The selectivity tests for sweat sensors of glucose (A), lactate (B), Na⁺ (C), and K⁺ (D). Data recording was paused for 30 s for the addition of each analyte in A-D.
Figure S18. The reproducibility of glucose (A), lactate (B), Na⁺ (C), and K⁺ (D) sensors (Eight functionalized electrode arrays for each kind of sensor).
Figure S19. The storage life of glucose (A), lactate (B), Na\(^+\) (C), and K\(^+\) (D) sensors (one to two months).
Table S1. The cost of a PS-VFTP based flexible electrode array.

<table>
<thead>
<tr>
<th>Consumable items</th>
<th>Amount (for two arrays)</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flash foam pad (diameter of 45 mm)</td>
<td>1</td>
<td>¥0.6</td>
</tr>
<tr>
<td>Tracing paper (diameter of 45 mm)</td>
<td>1</td>
<td>¥0.004</td>
</tr>
<tr>
<td>Transparency film (diameter of 45 mm)</td>
<td>1</td>
<td>¥0.006</td>
</tr>
<tr>
<td>PVDF filter membrane (diameter of 50 mm)</td>
<td>1</td>
<td>¥0.84</td>
</tr>
<tr>
<td>PDMS</td>
<td>2.4 g</td>
<td>¥1.9</td>
</tr>
<tr>
<td>SWCNTs</td>
<td>0.6 mg</td>
<td>¥0.37</td>
</tr>
<tr>
<td>Electric charge (1 kW h⁻¹ (vacuum drying and filtration process))</td>
<td></td>
<td>¥0.56</td>
</tr>
<tr>
<td>Total cost of a flexible electrode array</td>
<td></td>
<td>¥2.14/$0.33</td>
</tr>
</tbody>
</table>
Table S2. The comparison of sensitivity ($S$) of recently reported wearable sweat sensors for glucose, lactate, Na$^+$, and K$^+$.

<table>
<thead>
<tr>
<th>Ref.</th>
<th>$S$ for Glu (nA μM$^{-1}$ cm$^{-2}$)</th>
<th>$S$ for Lac (nA mM$^{-1}$ cm$^{-2}$)</th>
<th>$S$ for Na$^+$ (mV dec$^{-1}$)</th>
<th>$S$ for K$^+$ (mV dec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33.24</td>
<td>3112</td>
<td>64.2</td>
<td>61.3</td>
</tr>
<tr>
<td>2</td>
<td>89.12</td>
<td>2461</td>
<td>51.8</td>
<td>31.8</td>
</tr>
<tr>
<td>3</td>
<td>63.66</td>
<td>533.2</td>
<td>35</td>
<td>45.6</td>
</tr>
<tr>
<td>4</td>
<td>10.06</td>
<td>-</td>
<td>66.7</td>
<td>64.1</td>
</tr>
<tr>
<td>5</td>
<td>35.3</td>
<td>11400</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>58.2</td>
<td>41.5</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>19.6</td>
<td>14.6</td>
</tr>
<tr>
<td>8</td>
<td>6.93</td>
<td>-</td>
<td>-</td>
<td>62.2</td>
</tr>
<tr>
<td>9</td>
<td>238.2</td>
<td>428.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>58</td>
<td>-</td>
</tr>
<tr>
<td>This work</td>
<td>345.5</td>
<td>3169</td>
<td>60</td>
<td>58</td>
</tr>
</tbody>
</table>
REFERENCES


(7) Mou, L.; Xia, Y.; Jiang, X. Epidermal Sensor for Potentiometric Analysis of

