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Technical Note

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Point-of-Care Diagnoses: Flexible Patterning Technique for Self-Powered Wearable Sensors

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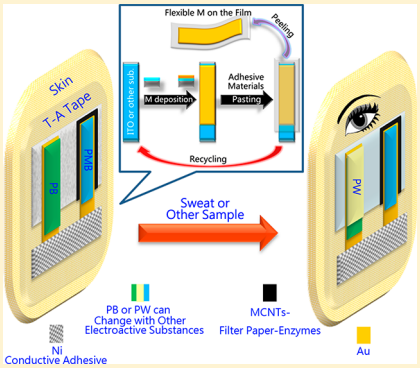
Supporting Information

ABSTRACT: This paper demonstrated the fabrication of a facile, low-cost, and self-powered platform for point-of-care fitness level and athletic performance monitoring sensor using electrochemical lithography method and its application in body fluid sensing. Flexible Au/prussian blue electrode was employed as the indicating electrode, where the color change was an indication of fitness level and athletic performance. A piece of Al foil, Au/multiwalled carbon nanotubes (MWCNTs)-glucose dehydrogenase, and Au/polymethylene blue-MWCNTs-lactic dehydrogenase electrodes were used for the detection of ionic strength, glucose, and lactic acid in sweat, respectively, which allows the sensor to work without any extra instrumentation and the output signal can be recognized by the naked eyes. The advantages of these sensors are (1) self-powered; (2) readily applicable to the detection of any electroactive substance by an electrochromic material; (3) easy to fabricate via two steps of EDP; and (4) point-of-care. By assembling the energy and sensing components together through a transparent adhesive tape, the proposed self-powered wearable biosensor exhibits superior performances, indicating its broad applied prospect in the point-of-care diagnoses.



Recently, the healthcare industry has experienced a major shift toward wearable devices[1−7] in personalized prescription,[8] health assessment,[4,5,9−11] forensic examination,[12] and motion recognition,[1,2] among which the epidermal sensor attracts much attention.[2−5,9,13] Such a device usually consists of electric circuit, electrodes, sensing components, power supplies and communication device or the combination of some of the above components on a flexible substrate. These interesting concepts have been well demonstrated in the recent important works, where a coil assembly was integrated on porous substrates, allowing the capacitive and colorimetric detection of sweat amount and its pH.[3,5] In order to maintain proper function of the epidermal sensor, biofuel cell was introduced on the skin to generate sufficient electrical power from human perspiration.[4] However, the biggest limitation of such wearable sensors so far is that the external instruments are required for data collection and processing. Moreover, the long-term stability of these devices is of a great technological challenge for the major deployment due to their considerably high cost.[2,4−6]

Based on the previous report,[14] we have demonstrated that the electrochemical lithography (EDP) method is facile, straightforward, and inexpensive in flexible thin film electrode fabrication, which makes the large-scale production feasible. Most importantly, the resulting flexible thin films were able to withstand repeated stress and could be used in various situations including flexible electric circuit and electrochemical interfaces. In this work, we fabricated a facile, low-cost, and self-powered monitoring platform for point-of-care fitness level and athletic performance by the EDP method. For this platform, flexible Au/prussian blue (PB) was employed as the indicating electrode, where color change is related to fitness level and athletic performance and can be easily recognized by the naked eyes, and a piece of Al foil, Au/multiwalled carbon nanotubes (MWCNTs)-dehydrogenase (GDH), and Au/polymethylene blue (PMB)-MWCNTs-lactic dehydrogenase (LDH) were used as counter electrodes for the detection of ionic strength, glucose, and lactic acid found in sweat, respectively, as shown in Scheme 1. Achieving this wearable, self-powered, and visually recognizable sensor, the fabrication of the flexible thin film of Au/electrochromic material, such as Au/PB and Au/PMB, is the key feature of our method for electrode fabrication. In addition, the constructed sensor with Au/PB electrochromic cathode (or other electrochromic anode or cathode) can be used for fitness level and athletic performance monitoring without any external instrumentation, and also exhibited excellent detection performances. Most

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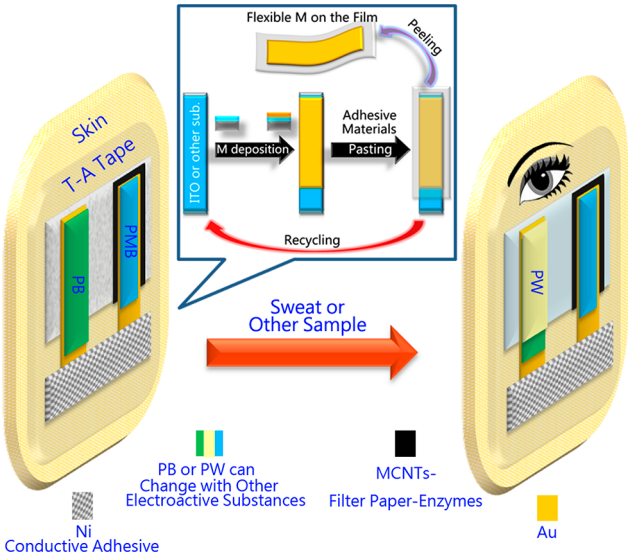
Scheme 1. Schematic Diagram of the Visual, Self-Powered, Flexible Sensor Based on the Electrochromic Sensing Strategy



importantly, it was a widely applicable method, meaning that it can be tailored to meet the demand of detection for different people. Thus, we believe that the proposed EDP strategy for flexible and patterned thin films, which we name lab-on-a-tape, may open up a new way for fabricating portable, disposable, self-powered, and wearable devices.

EXPERIMENTAL SECTION

Materials. Analytical reagent grade of all chemicals were used as received without any purification. β-D-glucose, NaCl, $FeCl_3$, ammonia, $K_3[Fe(CN)_6]$, and methylene blue (MB) was purchased from Beijing Chemical Reagent Company (Beijing, China). GDH, LDH, nicotinamide adenine dinucleotide phosphate (NADP), chlorauric acids, lactic acid, and urea were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI). A 175 mM NaCl solution (same ionic strength as in human sweat) and artificial sweat (0.5% sodium chloride, 0.1% lactic acid, 0.1% urea in deionized aqueous solution), artificial sweat with no lactic acid, and β-D-glucose solutions (10 mM in 0.1 M phosphate buffer, pH 6.0) were left at room temperature for 24 h before use. ITO-coated glass substrate with the resistance of 6 Ω /square was obtained from CSG Holding Co., Ltd. (Shenzhen, China). 102 filter paper was purchased from DongYang Industrial and Trading Co., Ltd. Whatman Chromatography Paper 3030−861 was obtained from Solaibao Biotechnology Co., Ltd. Al foil tape and the blue ink were obtained from the local grocery. Nickel conductive adhesive was purchased from Shenzhen, China. Transparent tape (T-A tape) was obtained from 3 M Co., U.S.A. All solutions were prepared with deionized water of 18 MΩ cm^{-1} by a Milli-Q system (Millipore, Bedford, MA).

Fabrication of Flexible Thin Film Electrodes. According to the previous work, Au and electrochromic material were successively deposited on the patterned ITO substrate and then peeled off by a transparent tape. In the electrochemical experiments, Ag/AgCl in saturated potassium chloride solution was used as reference electrode, against which all potentials used in this study were reported.[14] Au deposition was realized in 20 mM $HAuCl_4$ in deionized water using a double-pulse voltammetry (0.5 V ∼ 0.5 s and −0.2 V ∼ 1 s) for 180 cycles. The PB was deposited in the deposition solution (0.1 M KCl + 0.1 M HCl + 2.5 mM $FeCl_3$ + 2.5 mM $K_3[Fe(CN)_6]$) using potentiostatic method at 0.4 V for 80 s. The PMB was deposited in the presence of 0.5 mM MB mon-omer solution using 0.05 M phosphate buffer solution (pH 7.0 including 0.1 mM KCl) as supporting electrolyte under the potential sweeping between 0.6 and 1.1 V, and for 5 cycles.

The deposited PB on the Au layer acted as the indicating cathode for all three types of sensors explored in this study. For sensing the ionic strength in sweat, Al foil was used as sensor's anode, while Au and Au/PMB worked as anodes for glucose and lactic acid detection, respectively. The electric connection between the sensing cathode and anode was made using a Ni conductive tape. A filter paper (Whatman Chromatography Paper 3030−861) was pasted on the T-A tape as a self-driven sample collection cell. In addition, in order to improve the detection performance, GDH and LDH that incubated with MWCNTs modified filter paper were used for glucose and lactic acid sensing, respectively.

Semiquantitative Analysis. The semiquantitative analysis was performed in Adobe Photoshop. RGB mixed color mode was used to quantify the sensor color change by the sum of each distribution value of R, G, and B channels.

RESULT AND DISCUSSION

Wearable and Disposable Sensor for Fitness Level and Athletic Performance Monitoring. In order to demonstrate the application of the fabricated self-powered device based on the EDP strategy, we first examined the wearable device for sweat sensing or other body fluids (Scheme 1). To the best of our knowledge, there is no report on any wearable sensors for sweat or other body fluids monitoring that can work without an external power supply and signal acquisition instrument. As shown in inset in Scheme 1, to make such a device, indicator of PB deposited on Au (PB/Au), then Au/PB film was peeled off by the T-A tape, a piece of Al foil was pasted to the T-A tape placed next to the PB/Au electrode as the anode. Both electrodes were connected using a Ni conductive tape to form an electric circuit. Finally, a piece of filter paper was pasted on the T-A tape and acted as a sample collection cell. The schematic illustration of the fabricated device was displayed in Scheme 1, Figure 1a,b. The flexible sensor could be easily attached to the skin via the T-A tape. As shown in Figure S3a,b, the device was put on the arm of the volunteer, the color change was distinguishable and fast (a few seconds after the filter soaked wet) after 175 mM NaCl solution (same ioinic strength as in human sweat) was added, which could be recognized by the naked eyes. That was primarily because PB was reduced to Prussian white (PW, onset potential is ca. 0.35 V) at the cathode in the formed primary battery, where Al was oxidized to Al^{3+} (at ca. −1.663 V) at the anode, when the filter paper was saturated with the NaCl solution. This innovative design allows the sensor to work without any additional instrumentation, and the as-prepared device was able to generate sufficient energy to power the sensor from human sweat. Since the amount of sweat or other sample penetrated determines its discoloration, therefore, the filter paper thickness patterns can be designed simply by changing different types of filter paper employed (different capacities for sweat uptake) and the distance between the two working electrodes (Figures S1 and S2) to perform the exercise intensity monitoring according to the physical constitution of

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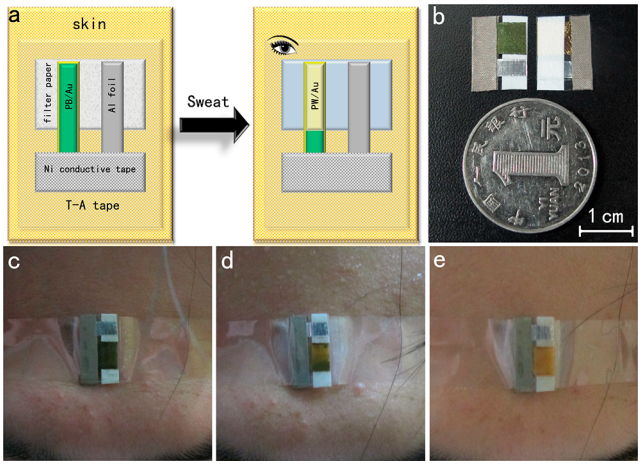

Figure 1. Schematic principle demonstration for the construction of the self-powered sweat sensing (a), photos of constructed sensor (b), and photos of time-dependent sensor on the volunteer's forehead during the exercise process, 0 min (c), after 29 min exercise (d), and after 32 min exercise (e).

users. How to determine this amount? The supplier can provide different filter paper thickness patterns for users. Then, the users can choose which kind of the test strips can be used according to the sweating and physical strength of themselves. Furthermore, the prepared sensor exhibited a long-term stability, as its ability for fitness and athletic performance monitoring was persistent after being stored in the air for more than three months.

Stepping further, the monitoring of exercise intensity and muscular exertion were achieved using this sensor. The sensor was applied to the volunteer's forehead, and the bare side of the filter paper (at the top of the sensor) was attached to his skin (Figure 1c). As shown in Figure 1d, after running for 29 min, the sweat started being adsorbed into the filter paper and the color of Au/PB electrode became partially yellow (color of Au). In another three minutes, the color of Au/PB electrode became completely yellow (Figure 1e). According to the previous reports, higher sweat-excretion rates within the same exercise time may indicate a lower fitness level.[3−5,7,11,15,16] Thus, the fitness level can be evaluated by the time needed to change the color of the Au/PB cathode using our design.

Detecting Biomolecules in Body Fluid Based on the Flexible Platform. Beside sensing the ionic strength in sweat, this facile design can also be used for the analysis of biomolecules in body fluid through the introduction of biological enzyme as bioanode, such as glucose oxidase (GOx),[3] GDH,[17] lactate oxidase (LOx),[4] and LDH,[18] which holds the bright prospect in the daily diagnostic application. Taking glucose sensing as an example, as also shown in Scheme 1, the whole process of visually recognizable glucose sensing device fabrication was the same as above, briefly, PB/Au electrode acted as cathode, MWCNTs adsorbed filter paper was used for the incubation of GDH, and then pasted on the flexible Au electrode acting as bioanode (Figure 2a−e). After adding 10 mM glucose (very fast adsorbed into the filter paper), the PB/Au anode was discolored to yellow in 37 min; meanwhile, obvious color change was not found after 60 min in the absence of glucose (Figure 2f), which was mainly attributed to the catalytical oxidation of glucose by GDH (−0.06 V), followed by the release of electrons to the Au/PB electrode, and finally leading to the reduction of PB to PW.

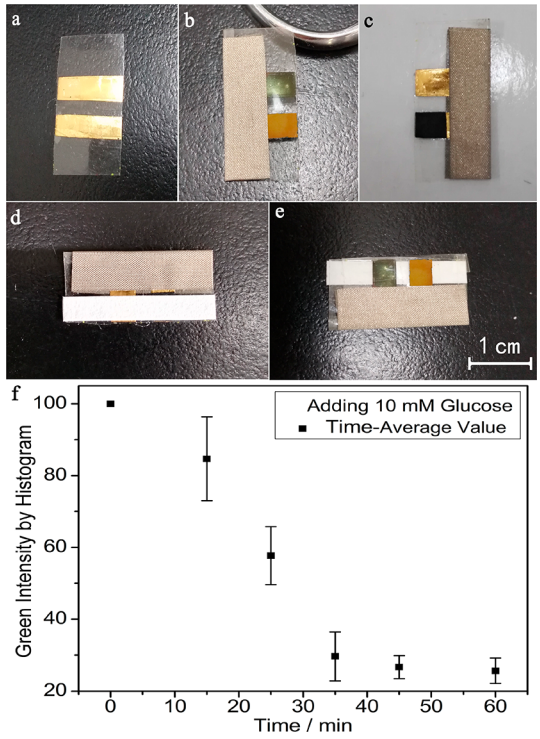

Figure 2. (a−e) Fabrication process of a complete wearable sensor for glucose detection: (a) flexible Au substrates used for fabrication of bioanode and Au/PB (photo taken form the back cover), (b) connection of the two eletrodes using Ni conductive tape (photo taken form the front cover), (c) adhension of the CNT-filter to the bioanode, (d, e) filter paper used for sample collection was integrated, photos taken form the front cover (d) and back cover (e), and (f) average value of three measurements based on the discoring time of glucose sensing strategy, the error bars represent standard deviation.

According to our previous work,[19] any oxidation reactions with an onset potential lower than that of PB reduction (onset potential ca. 0.35 V) can be monitored by the discoloring rate of PB, and thus, any electroactive substance, with an appropriate redox potential, can be indicated by an electrochromic material. The self-powered strategy is free from any kinds of external power source. Based on this strategy, the discoloring time or rate of PB cathode is related to the instrinsic property and concentration of species at the substrate, and the biocatalytic activity and amount of the biocatalyst. This lab-on-a-tape systems combine the simplicity, disposability, portability, and low-cost of tape-strip tests with the complex function and multiplex analysis of conventional lab-on-a-chip devices for analyte detection.

Apart from for the detection of glucose, we also fabricated a bioanode with LDH for visual detection of lactic acid in sweat. Just as discussed above, MWCNTs adsorbed filter paper was also used for the incubation of LDH to improve the electron-transfer, and PMB were electrodeposited on flexible Au electrode for catalyzing NADH oxidation to NAD^+ (Scheme 1). First, we confirmed that NADH can be converted to NAD^+ at the Au/PMB anode through its electrochemical oxidation. As sketched in Figure 3, without the addition of NADH, the peak associated with NADH oxidation did not appear; however, after adding 5 mM NADH, the Au/PMB anode exhibited the catalytic activity with onset potential of −0.04 V, due to the catalytic effect of PMB on the oxidation of NADH to NAD^+. As mentioned above, the reduction of PB to PW at

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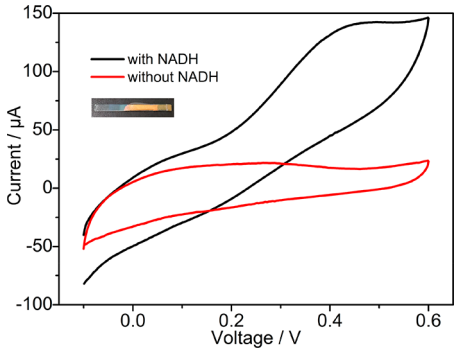

Figure 3. Cyclic voltammetry curve of NADH on the Au/PMB electrode due to the catalytical oxidation in 0.1 M phosphate buffer, pH 6.0.

the cathode occurred at about 0.35 V; therefore, a self-powered sensing platform can be performed for the detection of lactic acid visually.

Based on the results above, we expanded the flexible wearable sensor for point-of-care lactic acid detection in sweat. The detection was first performed in the artificial sweat. In Figure S5, the artificial sweat and artificial sweat with no lactic acid were used on two sweat sensors. In the artificial sweat with 0.1% lactic acid, the Au/PB indicating cathode was discolored from green to yellow (Figure S6); meanwhile, this observation was not found in the artificial sweat without lactic acid. The sensor was then applied to the volunteer's forehead and face by attaching the filter paper (at the top of the sensor) of the Au/PB bioanode to his skin (Figure 4I and II). After running for

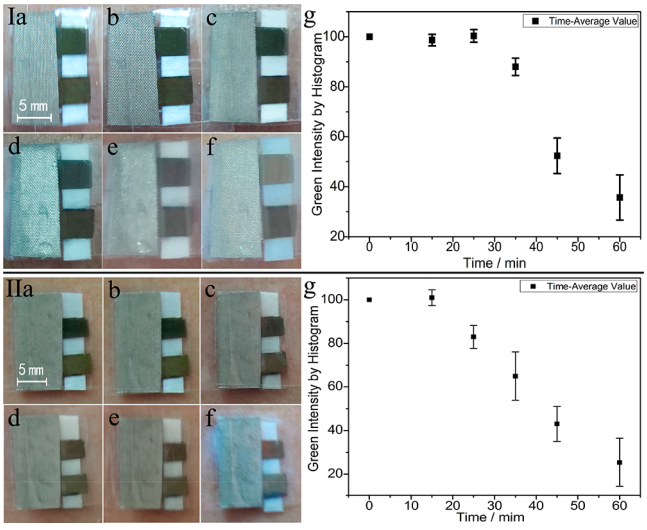

Figure 4. Wearable sensor for monitoring lactic acid generated on the volunteer's forehead (I) and face (II): (a) 0, (b) 15, (c) 25, (d) 35, (e) 45, and (f) 60 min. (g) The average value of three determinations based on the discoloring time of sweat sensors. Error bars represent standard deviation.

16 min, as the sweat started to adsorb into the filter paper, the Au/PB indicating cathode was gradually discolored to yellow on the sensor placed on the face. About an hour later, the color of Au/PB indicating cathode became completely yellow (Figure 4If and IIf), indicating the exercise intensity reached the maxima. Furthermore, it can be seen that the detection on the forehead was less distinguishable than that on the face (Figure 4If,g and IIf,g), which was likely attributed to a less amount of sweat produced on the forehead. In this design, the fitness level can be distinguished by the time that is needed to change the color of the Au/PB indicating cathode. Importantly, the self-powered strategy is free from any kind of external power source. Based on this strategy, the discoloring rate (or time) of Au/PB indicating cathode is related to the instrinsic property and concentration of the species at the substrate, that is, there would be no background signal theoretically, and this point-of-care strategy can provide more reliable and easier identification of the target compound. Along with the facile EDP strategy, we believe the point-of-care testing sensor is of great practical significance, and its mass production can be easily achieved based on the highly developed electroplating technique.

CONCLUSIONS

Using a low-cost EDP method, we developed a new and flexible Au/PB, Au/MWCNTs-GDH and Au/PMB-MWCNTs-LDH electrodes for self-powered and point-of-care fitness and athletic performance sensor. This innovative design allows the sensor to work without any extra instrumentation and the output signal can be recognized by the naked eyes. The advantages of these sensors are (1) easy to fabricate through two-step EDP method, electrodeposition and peeling, (2) self-powered without external power sources, (3) versatile and applicable to any electroactive substance with an appropriate redox potential, which can be indicated by an electrochromic material, (4) point-of-care. By integrating the energy and the sensing components together through a T-A tape, a self-powered biosensor was developed and tested to detect salt concentration, glucose and lactic acid found in sweat. The excellent performance for those target detections proved the broad applied prospect of our T-A tape in the point-of-care diagnosis and treatment. We believe the EDP strategy opens up a new way in designing the future portable, disposable, and wearable lab-on-a-tape sensors and holds bright promise for point-of-care diagnostics.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b02838.

Additional information about the paper selection, detection of ionic strength, glucose detection and artificial sweat test, detailed experimental methods, and parameters (PDF).

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Notes

The authors declare no competing financial interest.

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