Article

Journal of
Nanoscience and Nanotechnology
Vol. 20, 1495–1503, 2020
www.aspbs.com/jnn

A Wearable Biosensor Based on Bienzyme Gel-Membrane for Sweat Lactate Monitoring by Mounting on Eyeglasses

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A new enzymatic biosensor worn on eyeglasses has been developed for low-noise and noninvasive determination of lactate in human sweat during physical exercise. The Os (osmium)-complex, the electron mediator between the enzyme and the electrode, was first immobilized on a flexibly printed carbon electrode. Then, a gel membrane with the stereoscopic reticular structure of lactate oxidase and horseradish peroxidase was casted on the electrode to form the biosensor. Linearity of the biosensor was observed for up to 25 mM lactate in a phosphate buffered solution of pH 7.0. Chemical selectivity was evaluated by adding common interferent species such as ascorbic acid, glucose and uric acid to the lactate. The negligible current interference indicated excellent discriminatory selectivity of the biosensor. Applied to an analysis of the real sweat lactate dynamics of healthy subjects during cycling exercise, the amperometric profiles of the biosensors reflected changes in sweat lactate that depended on physical exercise intensity. Compared with other reported epidermal biosensors attached to the arm or leg, our biosensor not only exhibited a similar current change tendency but also rarely suffered from deformational interference due to their forehead measurement position. Such a successful application of real-time monitoring of sweat lactate means that eyeglass-bound biosensors hold considerable promise in the physical exercise and biomedical fields.

Keywords: Biosensor, Bienzyme Gel Membrane, Lactate Monitoring, Multiwalled Carbon Nanotubes, Sweat.

1. INTRODUCTION

Wearable/implantable biosensors hold great promise for the healthcare system since they enable the improvement of diagnostics, continuous monitoring, and maximization of the independence and participation of individuals [1–3]. Physiological monitoring could help in both the diagnosis and ongoing treatment of a vast number of individuals with neurological, cardiovascular and pulmonary diseases. Most of research activity on wearable sensors has focused on physiological or movement monitoring by physical signals. Recent developments in the field of chemical sensors or biosensors have allowed researchers to develop wearable biosensors to monitor the chemical constituents of the sweat on the epidermis of the person wearing the device [4–6].

In clinical diagnostics, lactate measurement is important in the monitoring of heart failure, respiratory insufficiency and metabolic disorders [7–9]. Lactate is also related to the stability, storage quality and freshness of food products such as beer, milk, wine and cider [10–12]. In sports medicine, blood lactate levels are used to monitor the maximum performance level of an athlete [13, 14]. During high-intensity exercises such as sprinting, cycling and gymnastics, the usual aerobic metabolism cannot satisfy the energy requirements of the human body. In such circumstances, the anaerobic process is started through the consumption of stored glycogen, producing energy and lactate as a by-product in muscle cells. This process is known as "glycolysis" or "lactate acidosis" and increases lactate levels in the blood [15, 16].

A high level of lactate in the body will cause lactic acidosis, which induces discomfort such as muscle

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aches, fatigue, and even coma or death. The monitoring of blood lactate is also suggested for the development of science-based athletic training programs. Lactate determination is performed by liquid chromatography or capillary electrophoresis [17, 18]. Although these techniques are highly accurate, they require laborious sample treatment and expensive instrumentation. Compared to their spectroscopy-based counterparts, electrochemical biosensors have been shown to be sensitive, selective and easy to use, and many lactate-selective electrodes have been reported [19–22]. Recently, many efforts have led to the development of biosensor strips based using lactate oxidase as a measure of lactate. However, this type of strip biosensor is commonly disposable and requires that a blood sample be obtained by sticking the finger or antebrachium [23, 24]. Continuous lactate curves are more meaningful than a single-point lactate measurement for athletic training. Therefore, the invasiveness and inconvenience of such blood tests are inherent drawbacks for obtaining a successive lactate curve of levels in athletes during physical exercise. As blood would have to be drawn repetitively from athletes, this procedure would hinder their normal training.

The lactate concentrations in the sweat have a good consistency with those in blood [16]. Therefore, it would be convenient to utilize noninvasive sweat sampling instead of invasive blood sampling for evaluating the physical ability of an athlete [25, 26]. Polliack and Jeddi reported that lactate in the sweat was considered a sensitive marker of some diseases of insufficient oxidative metabolism, such as myocardial ischemia, tissue viability, etc. [27, 28]. A noninvasive biosensor to detect lactate *in vivo* is a dream of many researchers who have made great efforts to achieve it through sweat or saliva sampling [29, 30]. However, these biosensors have mandatory requirements for sample collection at a specific time and then immediate laboratory analysis to obtain lactate levels. Such a labor-intensive measurement process is cumbersome and does not provide real-time dynamic results of lactate fluctuations in the body. With the development of personalized medical care, real-time and user-centric measurement technologies are highly desired for the efficient assessment of body information. Therefore, the improvement of noninvasive biosensors is still the focus of research efforts because of their intricate biological reactions and measurement processes.

Therefore, the ideal lactate detection approach should be noninvasive, convenient and continuous. Researchers have made many attempts to find a truly suitable method of noninvasive and continuous lactate monitoring. Body-worn sensing devices are highly desired for their ease of use. Recently, a wearable lactate biosensor based on an enzymatic electrode printed on an artificial sheet exhibited high sensitivity, stability and selectivity for using whole human saliva samples [31]. Khodagholy reported an electrochemical biosensor based on a transistor that could probably measure the sweat lactate levels via direct attachment to human epidermis [32]. A wearable multisensing patch for continuous sweat monitoring has also been explored using a flexible microfluidic platform attached to the waists of human volunteers [33].

Eyeglasses are widely used on various occasions as they offer vision calibration and considerable protection against eye injuries. The arms of the eyeglass frame are close to the skin of the head and maintain a rubbing against the skin, which forms an attractive platform on which to mount the miniaturized biosensor with protection against deformational interference. Herein, we present an example of an electrochemical biosensor that is hidden in eyeglasses to provide real-time, noninvasive and continuous monitoring of the sweat lactate of humans. The wearable sweat biosensing system is comprised of a printed thick-film transducer with electron mediators of the Os (osmium)-complex and a bioactive bienzyme gel membrane sensor. The three electrode pads were connected with the analytical device by three wires, one end of which was glued to electrode pads by silver colloid while the other end was connected to an analytical interface device. The biosensor exhibits good characterization of high sensitivity and selectivity due to its extremely low-potential detection and the exclusion of electroactive constituents of human perspiration. Eyeglasses with the mounted biosensor can be easily worn in the same manner as ordinary eyeglasses. More importantly, the lactate levels can be continuously measured without interruption since the biosensor keeps in touch with the skin at all times. Compared to earlier reports that used perspiration biosensors glued on the arm or leg, our eyeglasses biosensor can maintain a stable signal free from mechanical disturbance, which opens a new avenue for continuous assessment of dynamic changes in metabolites.

2. EXPERIMENTAL DETAILS

2.1. Reagents and Apparatus

Horseradish peroxidase (HRP), L-lactate oxidase (LOx), o-phenylenediamine (o-PD), phosphate buffered saline (PBS) tablets, L-lactic acid (LA), phenol, L-ascorbic acid (AA), chitosan, poly(ethylene glycol) diglycidyl ether (PEGDGE) and uric acid (UA) were purchased from Sigma Chemical Co., USA. Carboxy-functionalized multiwalled carbon nanotubes (MWCNTs) were purchased from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China). All reagents were analytical grade and were used without further purification or modification. The Os-complex mediator was prepared according to previously reported procedures [34, 35]. Deionized water was used to prepare all solutions.

Graphite paste, silver paste, and silver–silver chloride (Ag|AgCl) paste were purchased from Advanced Conductive Materials (Atascadero, CA). Stencils were patterned on 50 μm thick stainless steel. Insulating paste

(Polyurethane) was purchased from the Measurement Group UK Ltd. (Hants, UK). A CHI 660E Electrochemical Workstation (CH Instruments Inc., USA) was employed for the electrochemical measurements of the biosensor.

2.2. Fabrication of the Biosensor

The three electrodes of the biosensor were screen-printed on a clean polypropylene (PP) substrate material with the dimensions of $25 \times 5 \times 0.3$ ($L \times W \times H$) mm^3. The detailed process was as follows: First, silver paste was stirred and mixed thoroughly and then screen-printed on the PP substrate through a stainless-steel shadow mask for the conducting tracks. The printed silver tracks on the PP were dried for 30 min in an 80 °C oven. Subsequently, Ag|AgCl paste was printed on the end of a conducting track to form Ag|AgCl pads for use as reference electrodes. Graphite paste was finally applied to the remaining conducting tracks to form graphite pads as working and counter electrodes. After the screen-printing, the electrode arrays were kept at 80 °C for 1 h to anneal. A patterned adhesive shroud with insulating paste was adhered to the electrode arrays to define the reaction area and electrode pads for insulator electrodes (Fig. 1(B)).

Following the printing of the electrode transducers, the working electrode was modified with the reagent layer. MWCNTs were suspended in anhydrous alcohol (3 mg mL^{-1}) and sonicated for 3 hours for a uniform suspension. A volume of 2 μL of mixed solution containing Os mediator solution (0.5 mg mL^{-1}), MWCNTs, HRP (300 U mL^{-1}) and PEGDGE (0.25%) was dropcasted on the working electrode area. After drying overnight in a 4 °C refrigerator, the Os-complex mediator with HRP was modified on the working electrode surface by PEGDGE crosslinking through covalent bonds (Fig. 1(D)).

LOx was immobilized on the working electrode surface by electropolymeric entrapment in a poly(phenylenediamine) (PPD) film. This was accomplished using a 0.1 M phosphate buffer (pH 7.0) solution containing 10 mM o-PD, 5 mM sodium sulfate, and 500 U mL^{-1} LOx, which was purged with nitrogen for 20 minutes. The printable transducer was immersed in the polymerization solution; a cycling voltammetry from −0.5 to +1.2 V (vs. Ag|AgCl) at 0.05 V s^{-1} was subsequently applied for 50 cycles in order to grow the LOx-entrapped PPD film (Fig. 1(E)). Afterwards, the biosensor was rinsed three times and immersed in a 0.1 M phosphate buffer solution (pH 7.0) for 10 min to remove nonbound enzyme from the electrode surface. Following the cleaning process, the sensitive area of the biosensor was covered with 2 μL of a 0.5% w/w chitosan solution prepared in 1% w/w acetic acid. Finally, the biosensor was dried and stored in the refrigerator at 4 °C.

Figure 1(C) shows the chemical constitution of the modified working-electrode transducer and the cascade reactions occurring on the surface of the eyeglasses biosensor after adding the lactate. First, L-lactate was oxidized in the presence of LOx to pyruvate and H_2O_2. Second, immobilized HRP was oxidized by hydrogen peroxide to produce H_2O, which changed HRP from a reduced to an oxidized form. Then, the oxidized form of HRP was reduced by a divalent osmium (Os(II))-centered redox polymer.

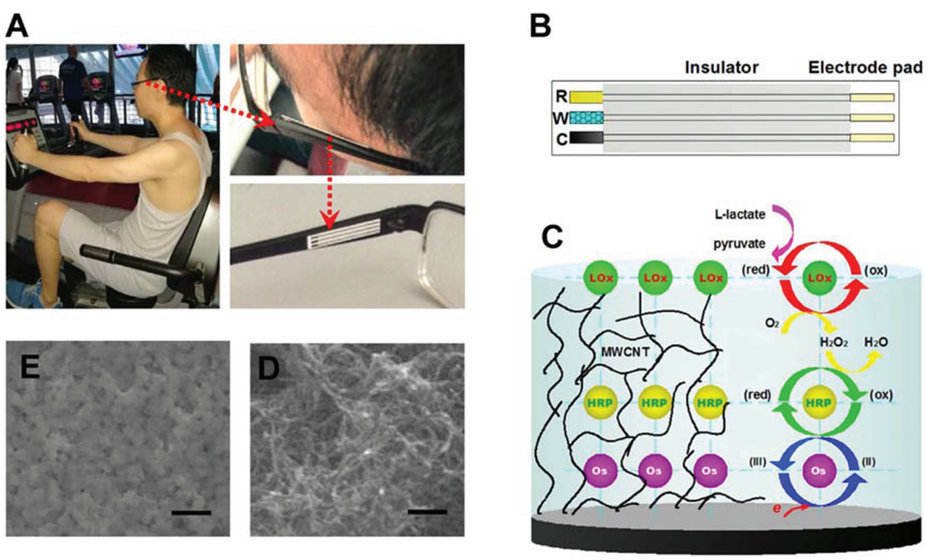

Figure 1. (A) Photograph of the biosensor integrated with glasses for lactate monitoring in human sweat. (B) Schematic diagram of the printable 3-electrode system of the biosensor. (C) Bienzyme gel-membrane layer and response mechanism of the biosensor mediated by the Os complex for lactate detection. Symbols: LOx (ox), LOx (red), HRP (ox) and HRP (red) are the oxidized and reduced forms of the aforementioned enzymes, respectively. (D) SEM image of MWCNTs with HRP and the Os complex immobilized on the electrode (scale bar: 200 nm). (E) SEM image after electropolymeric entrapment of LOx (scale bar: 500 nm).

The divalent osmium (Os(II)) polymer lost the electron and was oxidized to trivalent osmium (Os(III)) polymer, which changed from a reduced to oxidized form. The oxidized form of the trivalent osmium polymer (Os(III)) was then reduced by acquiring an electron from the working electrode, which changed the form from oxidizer to reducer. The last electrocatalytic reduction of Os(III) generated the analytical current that was proportional to lactate.

2.3. Performance of the Lactate Biosensor

2.3.1. Electrochemical Characterization in Buffer Solution

The electrochemical performance of the eyeglasses lactate sensor was evaluated *in vitro* using a 0.1 M phosphate buffer (pH 7.0) solution. After a 2-min incubation in the sample solution, the chronoamperometric current of the biosensor for lactate measurement was examined by applying the working electrode potential to 0 V (vs. Ag|AgCl) for 60 s. The applied potential was chosen based on the cyclic voltammetry of the layer-by-layer transducer [36, 37]. According to the cyclic voltammograms of the Os-complex mediator/enzyme membrane, 0 V (vs. Ag|AgCl) was suitable as the working potential because of the display of an onset current plateau phase at around that voltage for the oxidation of lactate, indicating that the Os-complex/LOx/Chit reagent layer offered low potential lactate oxidation. In this work, choosing 0 V (vs. Ag|AgCl) as the applied working potential could also reduce skin irritation during the prolonged monitoring of lactate levels. The stability determination of the biosensor for continuous lactate monitoring was carried out in 10 mM lactate at various intervals over a 6 h operation. Sensor specificity was examined in the presence of physiological levels of the relevant constituents of human sweat, namely, 10 μM ascorbic acid, 150 μM glucose, and 60 μM uric acid. The sensor was kept in 0.1 M PBS between successive measurements.

2.3.2. Human Sweat Measurements

Six volunteers (not professional athletes) with no heart conditions or diabetes were recruited for participation in the study. Two biosensors, with and without the LOx, were attached on the inside of two eyeglass frames to approve the selectivity of the biosensor. The positions of the sensors were adjusted manually to ensure the sensitive area could touch the skin (Fig. 1(A)). One end of three wires was connected to the contact pads of the biosensor, and the other was connected to the electrochemical analyzer. The output current of the biosensor was recorded using amperometry at a low potential of 0 V (vs. Ag|AgCl), which substantially reduces the possibility of electric shock due to the diminished electromotive force. The participants wore the eyeglasses and rode a stationary cycle for 30 min. The biosensor continuously detected the sweat lactate and its current profile reflecting lactate concentration was recorded in real-time. Sweat samples were also collected from foreheads during the cycling and then immediately analyzed. The *in vitro* measurement was used to estimate the effect of wearing biosensors for lactate measurement *in situ* as a comparison.

3. RESULTS AND DISCUSSION

3.1. Response of Biosensor for Lactate in Buffer Media

A voltammetry curve was employed to find the optimized operating potential of the glass sensor in the presence and absence of lactate in the buffer. The curve exhibited an onset current plateau phase at approximately 0 V (vs. Ag|AgCl) due to the oxidation of lactate. Therefore, the potential of 0 V (vs. Ag|AgCl) was selected for all subsequent chronoamperometric measurements. On the one hand, such a low potential illustrated the excellent lactate oxidation capability of the enzyme reagent layer; on the other hand, it reflected the efficient electron donor–acceptor interaction between Os and the electrode. The lactate concentration in human sweat depends on individual metabolism and physical performance and usually varies up to 25 mM [38]. The characteristics of the biosensor in response to lactate were subsequently investigated over the 0–25 mM range. Chronoamperogram curves of the biosensor were acquired with the increasing lactate concentrations. As shown in Figure 2, clearly defined amperometric curves of the biosensor responding to lactate in 0.1 M phosphate buffer (pH 7.0) show a tendency for the current to increase as lactate concentration increased. In the range of 0–25 mM, the current signal outputs by the biosensor exhibit a highly linear relationship with the measured lactate concentration. The sensitivity is 0.74 μA mM^{-1} and the correlation coefficient is 0.991 over the linear dynamic range, reflecting the excellent characteristics of the biosensor for lactate detection. The detection limit of the biosensor is the calculation of the standard deviation of the blank signal multiplied by 3 and divided by the sensitivity of the sensor, which is 0.04 mM. Such good performance is also deduced from the well-defined response to lactate and can thus illustrate that the biosensor enables effective detection of sweat lactate over the physiological range.

3.2. Characteristic of the Biosensor

A high level of stability is an important requirement for a biosensor used in practical epidermal applications due to the long periods. The stability of the biosensor was examined with repetitive measurements of 10 mM lactate over a 6-h period, wherein the response of the sensor was recorded at intervals of 20 min, 30 min and 60 min. As shown in Figure 3(A), chronoamperograms responding to the lactate measurements illustrated that the biosensor had highly reproducible results (RSD = 2.1%), displaying the long-term epidermal applicability.

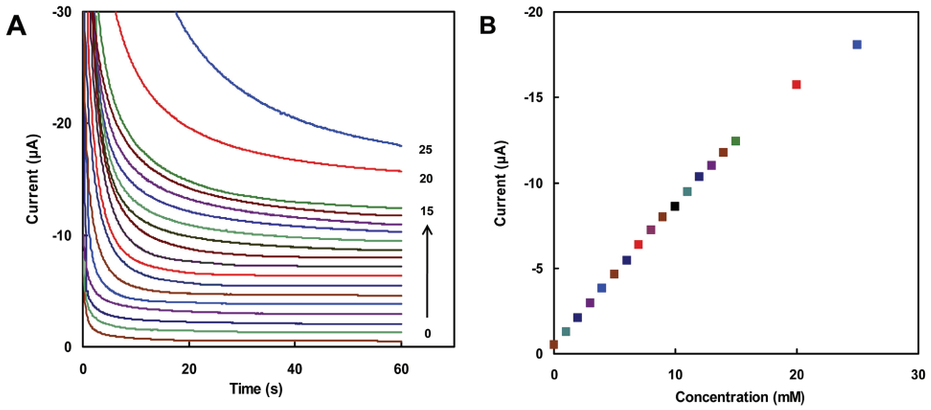

Figure 2. (A) Chronoamperograms obtained for increasing lactate concentrations in 1 mM increments up to 20 mM and 5 mM increments up to 25 mM in 0.1 M PBS. All experiments were performed with an E applied = 0 V (vs. Ag|AgCl) and a current sampling time of 60 s. The resulting calibration curve is shown in the inset. (B) Calibration plot using up to 25 mM lactate and based on sampling the current at 60 s.

The shelf life of the biosensor packaged in a vacuum and stored at 4 °C was also examined. The results showed that the biosensor retains its high reproducibility with an approximately 8% decline in sensitivity.

Raw sweat is a complex medium that consists of metabolites and electrolytes, such as ascorbic acid, uric acid, and glucose, which may disturb the determination of lactate and lead to inaccurate current readings. Thus, the biosensor was evaluated in the presence of these disruptors at physiological levels of sweat. The chronoamperometric curve of the biosensor response to 5 mM of lactate in the presence and absence of the abovementioned interferents is shown in Figure 3(B). The result clearly demonstrates that these interferents negligibly affect the lactate response, with less than 5% disturbed response compared to that with lactate. Such high selectivity of the biosensor benefits from the low operating potential, reasonable reagent layer, and the effective permselectivity behavior of the PPD layer.

The biosensor requires high operational repeatability during long operation periods for continuous monitoring of lactate. Additionally, since a progressive fluctuation of lactate takes place during intense physical activity, it is necessary to obtain changes in biosensor responses when the level of lactate fluctuates. The biosensor was subjected to successive changes in lactate concentration with three rinsing processes between changes of solutions. To get a stable signal, after changing the lactate level, we waited one minute before measuring the current signal. As shown in Figure 3(C), the biosensor signal was quite reproducible in three repeated carryover measurements that alternated lactate concentrations and low hysteresis effect when fluctuating lactate levels. Such good repeatability of the biosensor is a reflection of sufficient enzyme activity and efficient immobilization of the Os-complex electron mediator by a combined immobilization method in the three-dimensional network.

3.3. Performance of the Biosensor with Human Sweat

The biosensor experiments measuring human sweat were carried out following the evaluation of the biosensor in a synthetic buffer solution. The sweat samples were obtained from volunteers who were asked to stay in a room heated to 38 °C without strenuous exercise. The amperometric signal of the biosensor was detected when samples were spiked with lactate in concentrations ranging over 3–18 mM. Figure 4 displays the well-defined chronoamperograms of the biosensor for increasing lactate concentrations at 3 mM increments in human sweat samples. The calibration plot exhibits good linearity (slope, 0.810 mA mM^{-1}; correlation coefficient, 0.993) (shown in the inset of Fig. 4). Despite some potential interference of electroactive substances in the sweat samples, the biosensor still offers a well-defined and distinguishable amperometric response within the physiological levels of lactate.

The new glasses lactate biosensor was tested for practical application of monitoring lactate in human perspiration. Prior to epidermal lactate-sensing evaluation, the biosensors were adhered to the inner side of the arms of the glasses and adjusted to a location where the sensitive area just contacted the skin. Before wearing the glasses, both sides of the forehead skin of the volunteers were washed with deionized water and dried with a clean sterile towel in order to avoid any contamination. Real-time epidermal lactate levels of 6 volunteers were monitored utilizing the biosensors. Experimental measurements while individuals were exercising were conducted following a stationary cycling protocol. The lactate levels in volunteers' sweat were measured during cycling exercise for 30 min by connecting the three-electrode biosensor with an electrochemical analyzer.

To further verify the selectivity of the biosensor for monitoring lactate, two biosensors with the same production steps, with and without (serving as the control) LOx, were applied simultaneously on both arms of the glasses. Before use, the biosensors were soaked in PBS solution.

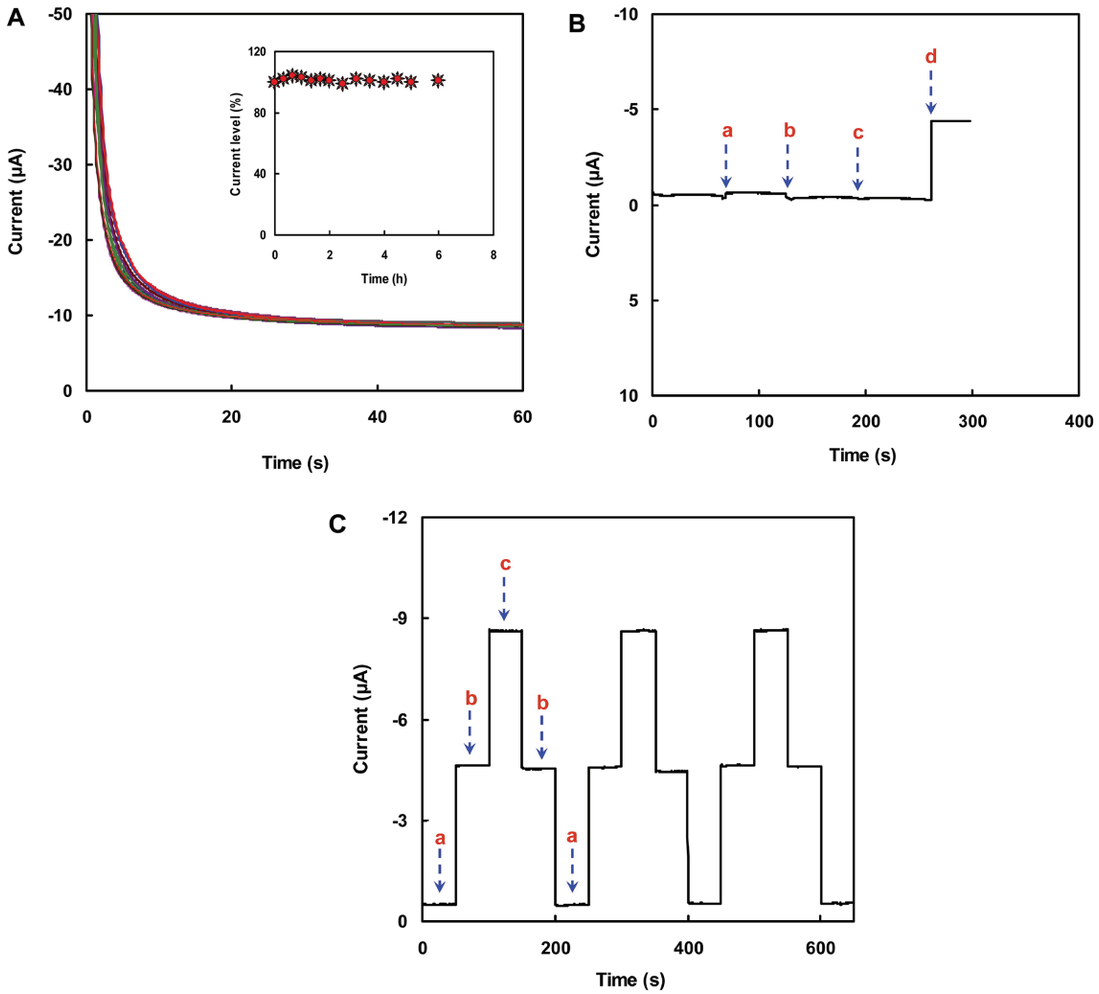

Figure 3. (A) Stability of the electrochemical response of the layer-by-layer biosensor to 10 mM lactate over a 6-h operation. Measurements were carried out in 20-min, 30-min and 60-min intervals. The inset is the corresponding current–time plot of the chronoamperometric response. The sensor was kept in 0.1 M PBS between such successive measurements. (B) Selectivity of the glasses biosensor. Response to 5 mM lactate in 0.1 M PBS in the presence of common electroactive physiological interferents: (a) 10 μM ascorbic acid; (b) 150 μM glucose; (c) 60 μM uric acid; and (d) 5 mM lactate. (C) Carryover test within (a) 0 mM; (b) 5 mM; and (c) 10 mM range in 0.1 M PBS. The biosensor was operated by waiting one minute before measuring when changing the lactate level. All other conditions were the same as those for Figure 2.

Figure 5 shows chronoamperometric profiles of the biosensors on one typical subject among the six volunteers. We can see that the current signal is characterized by low noise level, reflects the excellent quality of the biosensor and made good electrical contacts between the biosensor and epidermis without twist motion. Additionally, in the initial stage, the amperometric signals of both biosensors are overlaid and exhibit nonfaradaic current, reflecting no sweat and no lactate on the epidermis at this phase. With increasing physical exercise, the volunteer gradually began to sweat. The output of the LOx-containing biosensor (a, blue) rises to a higher level, which indicates the occurrence of a catalytic reaction toward lactate on the biosensor. Subsequently, as the sweat of the volunteer increased rapidly, the current signal of the LOx-containing biosensor also increases sharply. At approximately 1200 s, the output of the biosensor represented the lactate level in the sweat since the perspiration covered the whole surface of the sensitive area of the biosensor. Similar current curves were observed with the other volunteers, despite the lactate current rising at different time scales owing to their different aerobic capacity. During the final period (approximately the last 5 min), the volunteers cycled with a lower intensity, and their anaerobic respiration started to gradually drop. In particular, the considerable sweat secretion at this stage, which also diluted the lactate to some extent, resulted in the current signal turning from the peak in a downward trend. In contrast, the control "enzyme-free" biosensor worn by the subject displayed a slight variation in current (b, purple) throughout the exercise process, indicating the absence of lactate oxidation activity.

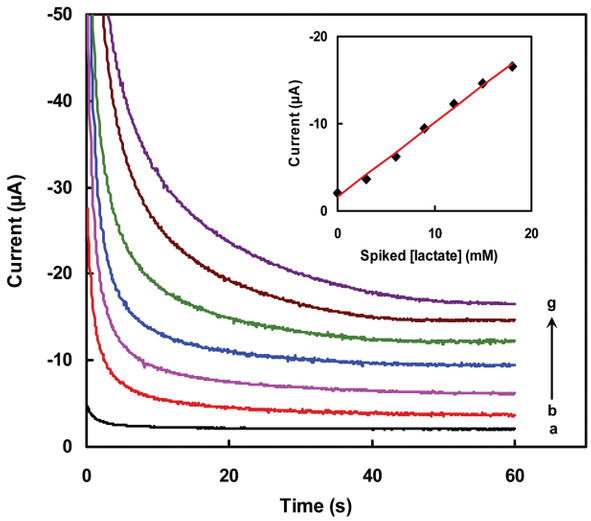

Figure 4. Chronoamperometric response for human sweat sample (a) spiked with 3–18 mM of lactate (b–g) at 0 V (vs. Ag|AgCl). The resulting calibration plot is shown in the inset.

Perspiration samples were also collected from the foreheads of the volunteers at approximately 16, 20, and 25 min during physical exercise, and lactate levels were examined using the new biosensor for further confirmation of the monitoring ability of the biosensor. The red triangles of Figure 5 are the lactate concentrations of the discrete samples, which indicate a better relationship between the *in vivo* response and the *in vitro* data (less than 10% deviation).

Table I shows the current signal of the biosensors on the six volunteers before and after physical exercise. We can see that the biosensors only exhibit the low initial current

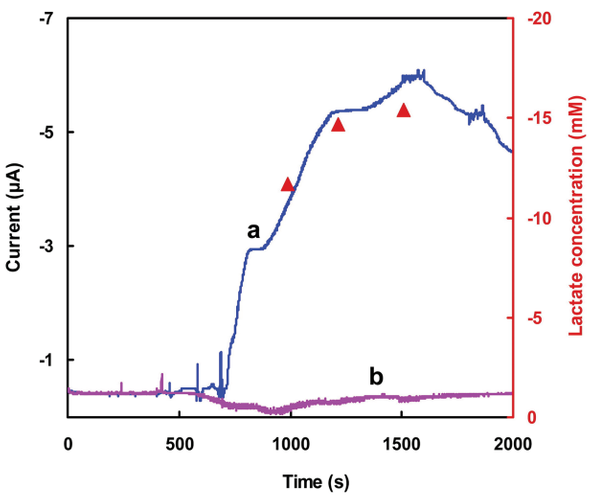

Figure 5. Real-time response of the LOx-containing (a) and enzyme-free (b) biosensors (left *y*-axis) during the exercise and the corresponding lactate concentrations (right *y*-axis); red triangles represent lactate concentrations in the sweat collected at prescribed times. Constant potential, 0 V (vs. Ag|AgCl); measurement intervals, 1 s.

Table I. Response current of the biosensor before and after physical exercise.

No.	I_1 (μA)	I_2 (μA)
1	0.51	6.72
2	0.69	4.35
3	0.42	5.68
4	0.74	8.22
5	0.57	0.62
6	0.48	5.94

Notes: I_1 is average current value in 200 ± 50 s before physical exercise. I_2 is average current value in 1200 ± 50 s after physical exercise.

(I_1) before the volunteers begin exercising. After approximately 20 minutes of high-intensity cycling exercise, volunteers showed signs of shortness of breath and sweating. Meanwhile, the current signal (I_2) of the biosensors also obviously increased, reaching about ten times that of the initial current. No. 5 is the singular case that did not conform to this rule; it is believed that the biosensor failed. Because of different individual fitness levels, even after the same intensity of exercise, the lactate level in sweat was also different, exhibiting different current values in Table I. However, the biosensor still reflects the dynamic changes of lactate levels in sweat, which are associated with the physical exercise intensity.

For epidermal measurements, the location of sample collection is a crucial factor and the concentration of L-lactate depends on the type of exercise [39]. Two epidermal biosensors were applied to different parts of the body in order to compare the real-time lactate concentration of different sampling locations among the volunteers. One attachment site was on the deltoid, and the other was on a pair of glasses (the eyeglasses biosensor as mentioned above). The volunteer was asked to mount a stationary cycle and begin cycling at similar intensity. The responses of the biosensors for the same volunteer at 5 min intervals over a 30 min period are graphically displayed in Figure 6(A). After an adequate rest, three replicates from the same volunteer were used to generate the calibration curve. As shown, the sample from the deltoids showed higher lactate levels and peaked at higher maximum values, but the response of the biosensors illustrated that samples collected from deltoid and forehead had the same trend. Figure 6(B) shows the typical chronoamperometric profiles of the two lactate biosensors during physical exercise. The profile of the biosensor glued on the deltoid (a, red) displayed greater noise level than that of the eyeglasses biosensor (b, blue), reflecting the low quality of the current transduction and unstable electrical contact due to epidermal deformations on the deltoid. The epidermis of the muscle usually experiences deformations, such as bending, stretching, and twisting stresses, when humans exercise. Epidermal biosensors, since they were glued on the epidermis directly, bore disfiguration similar to the skin due to the epidermal deformations, which often

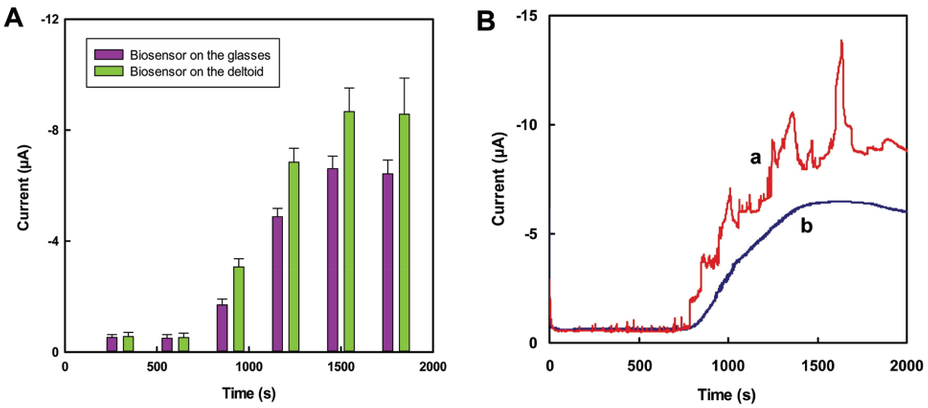

Figure 6. (A) Response of the biosensors on eyeglasses (purple) and on the deltoid muscle (green) at 5-min intervals over a 30-min period for one subject. (B) Typical chronoamperometric profiles from the biosensor glued to the deltoid (a, red) and on eyeglasses (b, blue) during the exercise regimen.

lead to an evident deviation of the sensor response. Our biosensors are glued on the arm of eyeglasses and its sensitive area contacts both sides of the skin of the forehead, which barely experience deformation during exercise, so such glasses biosensor less suffer from this interference.

4. CONCLUSION

We have developed an effective epidermal electrochemical biosensor that can continuously monitor lactate levels in sweat during physical exercise. The biosensor was designed through the use of a bienzyme gel membrane casted on a PP substrate and was flexibly attached to the arm of a pair of eyeglasses. In the buffer matrix, the biosensor showed a highly sensitive, selective, stable, and reproducible lactate response. The results of the epidermal experiment demonstrated that the biosensor is able to monitor lactate levels in sweat, which closely follows exercise intensity. Compared with traditional lactate determination by blood collection, the epidermal biosensor is noninvasive, continuous and easy to use. The results were comparable to those of other reported epidermal biosensors on the arm or leg doing the same type of experiments. Our biosensor suffered less from deformational interference due to the forehead measuring position. Future efforts will be aimed at integration with the glasses and signal acquisition system with wireless transmission. Moreover, the correlation of lactate levels between perspiration and blood also need to be explored in the future. The new concept of using glasses for epidermal biosensing can easily be expanded to the monitoring of other metabolites in sweat and applied to the assessment of athletic performance or to other uses in the clinical healthcare domain.

Acknowledgments: This work was supported by the National Natural Science Foundation of China (61801256), Shandong Province Key research and development plan (2018GGX109016) and Beijing Natural Science Foundation (4182075).

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Received: 19 November 2018. Accepted: 9 February 2019.