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# Wearable electrochemical biosensor based on molecularly imprinted Ag nanowires for noninvasive monitoring lactate in human sweat

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ABSTRACT

We proposed a wearable electrochemical biosensor based on Ag Nanowires (AgNWs) and molecularly imprinted polymers (MIPs) prepared on the screen-printed electrode for the noninvasive monitoring of lactate in the human sweat. The MIPs were prepared by electropolymerization of 3-aminophenulboronic acid (3-APBA) with imprinted lactate molecule on the AgNWs-coated working electrode. The MIPs-AgNWs biosensor revealed high sensitivity and specificity for the detection of lactate from $10^{-6}$ M to 0.1 M, with the detection limit of 0.22 μM. Furthermore, the sensors presented high stability and reproducibility with sensitivity recovery of 99.8% ± 1.7% during 7 months storage in a dark plastic box at room temperature. In addition, the flexible electrodes also showed stable electrochemical response after been bended and twisted for 200 times, respectively. Such MIPs-AgNWs biosensor was attached on volunteers' skin for the noninvasive monitoring of the perspiration lactate *in vivo*. The wearable electrochemical biosensor provides feasibility in near future for the evaluation of human sweat in the military, sports and biomedical fields.

## 1. Introduction

Wearable sensors have attracted increasing interest for the monitoring of human health conditions and physical performance, due to their flexibility which allows for the contact with soft epidermis and their portability for continuous monitoring of physiological status [1–3]. Early efforts in this field focused on monitoring the physical signals, such as skin temperature [4], heart rate [5], blood pressure [6], respiration rate [7], and bodily motion [8]. However, in order to comprehensively analyze individual's health condition, wearable chemical sensors and biosensors have been developed in recent years to obtain the chemical constituents information from human epidermis [9]. Wearable epidermal sensors for the analysis of human sweat have sprung up such as a series of tattoo biosensors [10,11]. It has been employed for the detection of lactate [10,12,13], glucose [14,15], alcohol [16], and urea [17] levels in the human perspiration. Among these chemicals, lactate is considered as a key component for evaluating the athlete's performance, particularly in participating in the endurance and high strength activities.

Generally, L-lactate as metabolite is a product of anaerobic metabolism of glucose in muscles. The increase amount of lactate production may result in cell acidosis and the disruption of the muscles performance. Lactate has been considered as one of the excellent biomarkers for tissue oxygenation, which is used for assessing human's physical performance [18]. Moreover, sweat lactate is an important product of eccrine gland energy metabolism in body fluids. The production of sweat lactate is positive correlation to the exercise intensity. Lactate concentration in the sweat could rise up to 25 mM in normal people during extensive exercise [10]. This threshold limits the person to continue the physical activity. Otherwise, the imbalance of the lactate production and its clearance may cause lactic acidosis. More serious is that the increase of lactate production could result in anaerobic metabolism, such as pulmonary embolism, or hemorrhagic shock. Therefore, the monitoring of sweat lactate is a more convenient and noninvasive way for the investigation of physical performance as compared with invasive blood sampling detection [19].

For the perspiration detection, electrochemical sensors based on enzyme catalysis [20,21] and molecular imprinting [22–26] are

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exclusively applied due to their simplicity, high sensitivity, intrinsic quantification and low electrical power requirements. Most lactate wearable sensors employed enzymatic (*e.g.*, lactate oxidase or lactate dehydrogenase) reaction to catalyze lactate for obtaining electrochemical response signal [27,28]. However, the activity of enzyme is affected by the factors of temperature, pH, and ionic strength. Moreover, enzyme may be degraded under unproper storage and usage which affected its sensitivity and the ability for long-time monitoring of lactate [29]. Instead, molecularly imprinted polymers (MIPs) have provided the advantages of low cost, good reproducibility, high stability and specificity. Recently, numerous monomers have been developed as the crosslinker for the electropolymerization of MIPs, such as pyrrole [30], poly(3,4-ethylenedioxythiophene) (PEDOT) [31], 3-aminophenylboronic acid (3-APBA) [32]. Pereira and co-authors have electropolymerized o-phenylenediamine (o-PD) with reduced graphene oxide and gold nanoparticles, and imprinted lactate on the film for lactate detection [26]. Such a sensor achieved excellent selectivity for the detection of lactate in sugarcane vinasse and the recovery values ranged from 97.7 to 104.8%. In addition, the MIPs prepared by the electropolymerization of 3-APBA in sulfuric acid solution have been employed for the detection of lactate with limit of detection (LOD) of 1.5 mM and applied for sweat sample detection [12]. Up to now, few works have been reported with wearable MIPs for the on-body detection of lactate in human sweat.

Here we report a flexible electrochemical biosensor based on Ag nanowires (AgNWs) and MIPs for the monitoring of lactate in the perspiration. AgNWs have the advantages of excellent electrical conductivity and good flexibility. The sensor is implemented by a screen-printing technique on a flexible substrate for the monitoring of lactate in the epidermal biofluids. The MIPs were firstly prepared by electropolymerization of 3-APBA with imprinted of lactate in PBS on the AgNWs coated carbon working electrode. After that, the lactate molecules were rinsed from MIPs to leave the specific binding sites for the detection of lactate in sweat. As shown in Fig. S1, the new epidermal lactate sensor consists of MIPs-AgNWs coated carbon working electrode, Ag/AgCl reference electrode and carbon counter electrode. The sensitivity, specificity and reproducibility of MIPs-AgNWs biosensor were investigated on the monitoring of sweat lactate. In addition, the lactate sensor was applied on the volunteers' epidermis for the monitoring of lactate *in vivo* based on the measurement of differential pulse voltammetry (DPV) response. We believe the lactate biosensor could be applied as a noninvasive flexible wearable device for the analysis of people's perspiration.

## 2. Experimental

### 2.1. Regents and instruments

L-lactic acid, (L118493, 98%) and ascorbic acid (A103533, 99%) were bought from Aladdin Co., Ltd (China). 3-aminophenylboronic acid (3-APBA, A823222, 98%), D-(+)-glucose (D810588), urea (U820349, 99%), uric acid (U820317, 99%), pyruvic acid (P815676, 98%), Calcium Chloride Anhydrous (C805228), Potassium Chloride (P816348, 99.5%), Sodium Chloride (S805275, 99.5%), Acetic acid (A801295, 99.5%) were all purchased from Macklin Co., Ltd (China). $AgNO_3$ (99.8%) was got from Shanghai Qiangshun Chemical Reagent Co., Ltd. Ethylene glycol (EG) and N-methyl pyrrolidone (NMP) were purchased from Macklin. Poly(vinylpyrrolidone) (PVP, average molecular weight of 55000, PVP-55000), PVP-1300000, and PVP-360000 were bought from Sigma-Aldrich. PBS powder (PH1403, pH = 7.2 ~ 7.4, 10 mM) was obtained from Scientific Phygene. All reagents were used as received without further purification.

SEM images were measured by a field emission scanning electron microscope (model SU8000, HITACHI, Japan). All electrochemical measurements were implemented with an electrochemical analyzer (model CHI660E, CH Instruments, Shanghai, China). A three-electrode system was employed in the experiment with a carbon working electrode, an Ag/AgCl reference electrode and a carbon counter electrode, which were made by the screen-printing technology and purchased from Shengtianfeng Co., Ltd (China). Polyethylene terephthalate (PET, thickness 0.075 mm) was employed as the flexible substrate of the electrodes.

### 2.2. Preparation of silver nanowires ink

AgNWs were synthesized by reducing $AgNO_3$ with PVP as surfactant in the EG solvent as reported elsewhere [33]. Briefly, 0.168 g PVP-55000 and 0.1625 g PVP-360000 were firstly dissolved into 44 mL of EG. Then, $FeCl_3$ (1.2 mM in 5 mL EG) and 0.54 g $AgNO_3$ (in 6 mL EG) solution were added into the above PVP solution for 1 min. The reaction process was kept in oven at 130 ℃ for 150 min. After cooled down, the product was centrifugated and re-dispersed in ultrapure water repeatedly, and then collected with the precipitate for AgNWs ink.

For the preparation of AgNWs ink, 3 *g* PVP-1300000 was dissolved in a mixture of 10 mL glycerol, 40 mL NMP and 50 mL EG under a vigorous stirring for 6 h. Subsequently, 60 mg AgNWs product was dispersed into 12 mL PVP-1300000 mixture solution under a stirring for 1 h at room temperature. Finally, AgNWs ink was centrifuged and re-dispersed in ethanol at the final concentration about 5 mg/mL which was stable up to several months under sealing in the dark place.

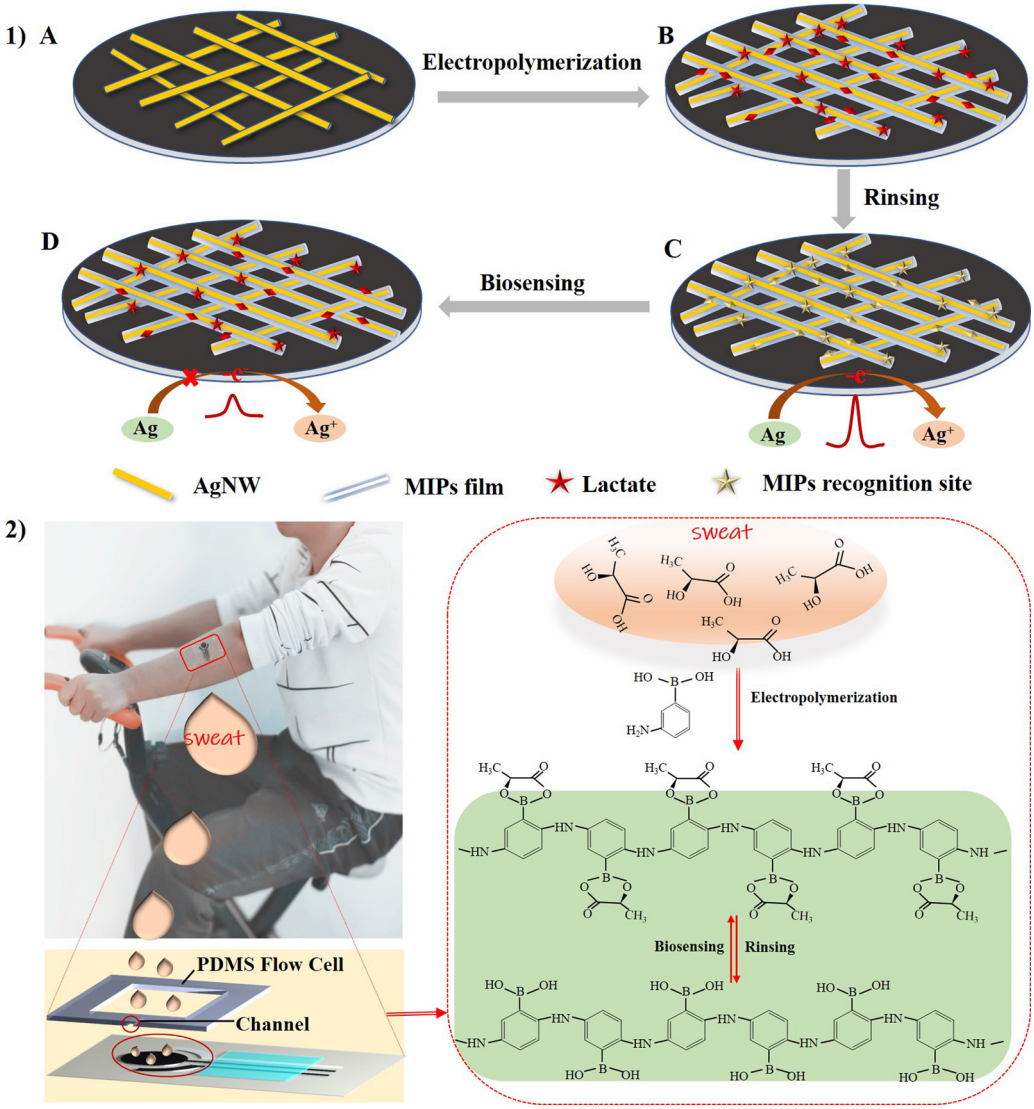
### 2.3. Fabrication of MIPs-AgNWs biosensor

Firstly, 20 μL of the prepared silver nanowires (5 mg/mL) were spin-coated on the carbon working electrode at rotated speed of 500 rpm (Scheme 1A). Then, the three-electrode system was dried at 65 ℃ for 1 h. The morphology of carbon working electrode was shown in Fig. 1A. The MIPs was electropolymerized on the AgNWs coated carbon working electrode (Scheme 1B). Briefly, 0.012 g of lactate, 0.0363 g 3-APBA and 0.1095 g sodium chloride were dissolved in 10 mL, 10 mM PBS solution (pH = 7.4). After kept quiet in the dark place with pre-polymerization for 4 h, the reaction solution was added into a home-made PDMS cell (with a volume of 0.9 mL) in contact with the three-electrode system based on PET substrate. The electropolymerization was carried out by cyclic voltammetry (CV) scan at a scan rate of 50 mV/*s* in the potential range between −0.4 V and +0.4 V for 30 cycles. The electrodes were rinsed with 8% acetic acid in PBS for 2 h to remove the lactate molecule from MIPs and form the recognition cavities (Scheme 1C). The lactate molecules could specifically bind to the recognition sites of MIPs for the detection of lactate (Scheme 1D). For a blank control, a non-imprinted polymers (NIPs) electrode was prepared using the same conditions excluding lactate in reaction solution.

To achieve the highest current response after removal of lactate molecules from MIPs, the MIPs electropolymerization was optimized by changing the number of CV scan cycles (5 ~ 50) and the concentration of monomer 3-APBA (13 ~ 52 mM). Furthermore, the time-dependent binding kinetics of lactate to the MIPs-AgNWs were also investigated for 35 min in order to gain the optimal adsorption time.

### 2.4. In vitro evaluation

CV, I-T, EIS (electrochemical impedance spectroscopy) and DPV measurements were carried out to characterize the process of the MIPs polymerization, washing and adsorption dynamics. The MIPs and NIPs adsorption properties were characterized by DPV measurement in PBS. DPV was used to investigate the lactate levels with an applied potential ranged from −0.2 V to +0.3 V, with pulse width of 0.05 s, amplitude of 0.05 V, sampling width of 0.0167 s and pulse period of 0.5 s. The peak current intensity of MIPs-AgNWs functionalized electrode was recorded as $I_0$ and $I_r$ for the MIPs before and after the removal of template molecules, respectively. The peak current changes $\Delta I_1 = I_r - I_0$ indicated the maximal response changes upon the removal of template molecules




**Scheme 1.** (1) The scheme shows the fabrication of the MIPs-AgNWs electrochemical biosensor for the epidermal monitoring of lactate. (A) AgNWs spin-coated on the carbon working electrode (WE). (B) Lactate MIPs-AgNWs on WE. (C) Lactate imprinted recognition cavities of lactate MIPs-AgNWs electrochemical biosensor. (D) Lactate biosensing with imprinted recognition sites in PBS solution or human sweat. (2) A screen printed three-electrode biosensor chip applied on a male volunteer's arm, and the principle of MIPs' formation and biosensing.

from the MIPs. Whereas for the detection of lactate, the peak current intensity was recorded as $I_a$ upon the incubation of MIPs-AgNWs biosensor in a series of lactate in PBS solution. The sensor response for the detection of lactate was characterized according to the peak current changes $\Delta I_2 = I_r - I_a$. Similarly, the NIPs-AgNWs electrode was investigated as control.

The interference experiments of MIPs-AgNWs and NIPs-AgNWs were also investigated in different interferent molecules at their physiological normal levels. The interferent molecules included glucose (0.17 mM), urea (10 mM), pyruvic acid (0.18 mM), uric acid (0.05 mM), NaCl (30 mM), $CaCl_2$ (5 mM) and $NH_3{\cdot}H_2O$ (5 mM) in PBS solution, respectively [12,17]. The peak current responses of MIPs-AgNWs and NIPs-AgNWs biosensor were recorded before and after the measurement of different interferent molecules.

### 2.5. The flexible performance test

For the practical applications, strains (bending and twisting) were applied on the electrode to investigate the effect of mechanical deformation to their DPV current peak changes. The electrode DPV were measured before and after bending (180° inward bend) or twisting (rotational bend) for 20 ~ 200 times. The influence of mechanical deformation on the electrochemical properties was evaluated as the peak current changes before ($I_b$) and after the applying of strains ($I_s$).

### 2.6. Epidermal lactate sensing

The epidermal lactate measurement was carried out on six healthy volunteers who are 3 males and 3 females between the age of 24 and 30. They have no heart disease, kidney injury or uremia. The current response $I_r$ of rinsed lactate from MIPs sensor was recorded before the epidermal measurement. Then, the biosensor with PDMS flow cell was fixed on the volunteer's arm (Scheme 1(2)). The PDMS was replicated from a mold to have the cell length of 15 mm, width of 10 mm, and thickness of 200 μm, with a small channel on the PDMS (length of 2 mm, width of 1 mm and thickness of 100 μm). Note that the channel was immobilized upward on the volunteer's arm to allow the overflow of sweat after fulfill the cell. The DPV responses were recorded each 5 min during an indoor exercise on treadmill for half an hour. The DPV peak current value measured as $I_a$ and the current changes were

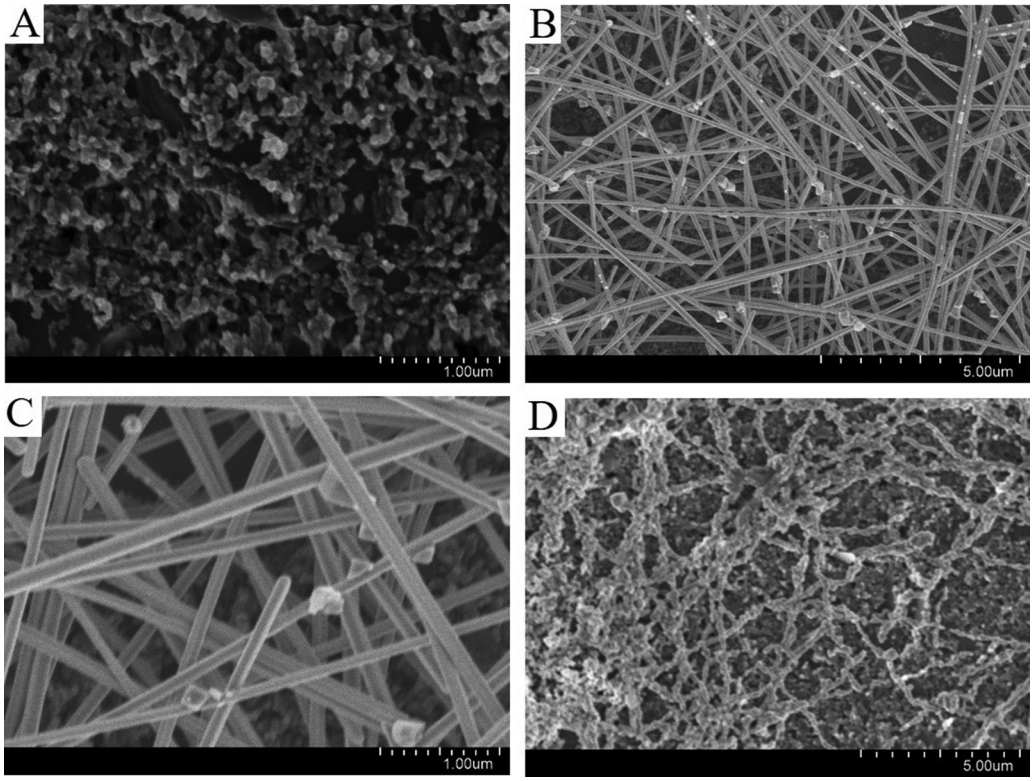



**Fig. 1.** SEM images of (A) the screen-printed carbon working electrode, (B and C) AgNWs coated carbon electrode and (D) after electropolymerization of MIPs on the AgNWs coated carbon electrode.

estimated as $\Delta I = I_r - I_a$. According to the concentration dependent current changes, the sweat lactate concentration was estimated accordingly.

## 3. Results and discussion

### 3.1. Preparation and characterization of MIPs-AgNWs

The lactate MIPs-AgNWs biosensor has been established by electropolymerization of 3-APBA with imprinted lactate on the carbon working electrode. The morphologies of the carbon working electrode and AgNWs have been characterized by SEM images. Fig. 1A shows the porous structure of the carbon working electrode. In Fig. 1B and C, it was clearly shown that the AgNWs distributed randomly and not aggregated on carbon working electrode. The diameter and length of AgNWs were observed around 120 nm and 15 μm respectively by SEM images (Fig. 1B and C). Note the small clusters as observed on the AgNWs in Fig. 1B and C might be the Ag nanoparticles formed during the reduction of $AgNO_3$ in the synthesis. The surface morphology of AgNWs became rough after the formation of MIPs on the AgNWs coated carbon electrode (Fig. 1D). It indicated that MIPs were electropolymerized on the surface of AgNWs due to the higher conductivity of AgNWs as compared with carbon electrode. The principle for the formation of MIPs was shown in the Scheme 1(2), that the derivative of polyaniline was formed through the electrophilic *para*-substitution relatively amino group [34]. Moreover, boronic acid residue of 3-APBA, as weak electron acceptors, forms a complex with lactate in weakly acidic solutions. After that, due to the negative charge allocated at the boron atom, poly(3-APBA) is converted into an electron donor group.

The electropolymerization of MIPs was performed by CV scan for 30 cycles with scan rate of 50 mV/s between −0.4 V and 0.4 V on the naked carbon electrode (Fig. S2A and B) and AgNWs coated carbon electrode (Fig. 2A and B), respectively. As shown in Fig. 2A and B, the current intensity decreased during the CV scan due to the formation of MIPs on the AgNWs coated electrode which weakened the conductivity of the electrode [35]. For instance, the oxidation current intensity of AgNWs decreased from $3.78 \times 10^{-4}$ A to $1.36 \times 10^{-4}$ A after the polymerization of MIPs on the electrode (Fig. 2B). In addition, compared with the MIPs electropolymerization on the carbon electrode (Fig. S2B), the one prepared with AgNWs showed two order of magnitudes higher current intensity after 30 cycles CV scan due to the high conductivity of AgNWs as shown in Fig. 2B.

### 3.2. Optimization of MIPs polymerization

The MIPs electropolymerization was optimized to obtain the highest signal changes after the removal of lactate molecules from MIPs, by varying the number of CV cycles and the concentration of 3-APBA. The DPV peak current changes ($\Delta I_1$) was defined as the measured peak current differences before ($I_0$) and after the removal of template molecules lactate ($I_r$), *i.e.* $\Delta I_1 = I_r - I_0$. Generally, the polymer thickness was optimized by controlling the number of CV cycles in the electropolymerization process. In Fig. S3A, the $\Delta I_1$ increased from the CV scan of 5 cycles to 30 cycles, and decreased after increase the CV scan from 30 to 50 cycles. This should be ascribed to the formation of a thick non-conductive polymer which increased the lactate adsorption capacity but also hindered the electron transfer to the electrode surface. Meanwhile, different concentrations of monomer 3-APBA in the reaction solution were investigated for the MIPs preparation. It was shown that the peak current achieved the highest response at the monomer concentration of 26 mM (Fig. S3B).

Additionally, the time-dependent binding kinetics of lactate to the MIPs-AgNWs were also investigated. The peak current intensity was increased gradually and reached equilibrium after 20 min incubation with $10^{-2}$ M lactate in PBS (Fig. S3C). Therefore, the adsorption of lactate for 20 min was considered as the optimal incubation time.

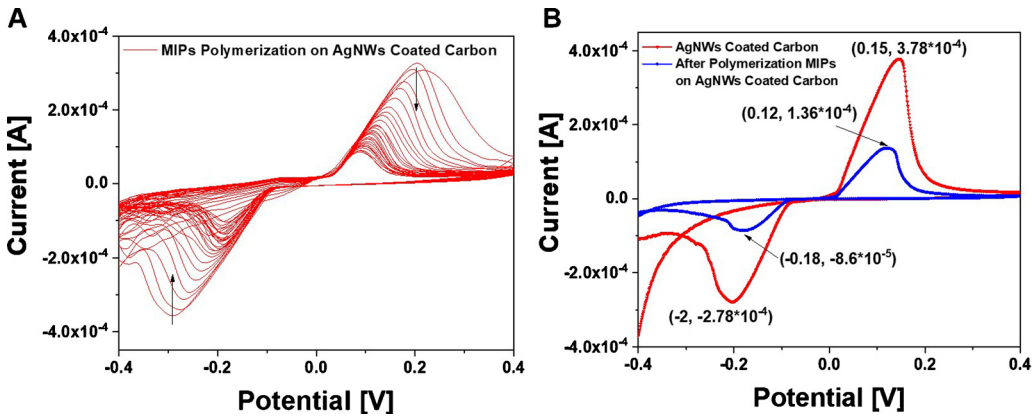



**Fig. 2.** (A) CV voltammograms recorded during the electropolymerization of lactate MIPs on the AgNWs coated carbon electrode (30 cycles, sweep rate 50 mV/s) in reaction solution. (B) CV voltammograms measured before and after the polymerization of MIPs on the AgNWs coated carbon electrode in the PBS (pH = 7.4, 10 mM).

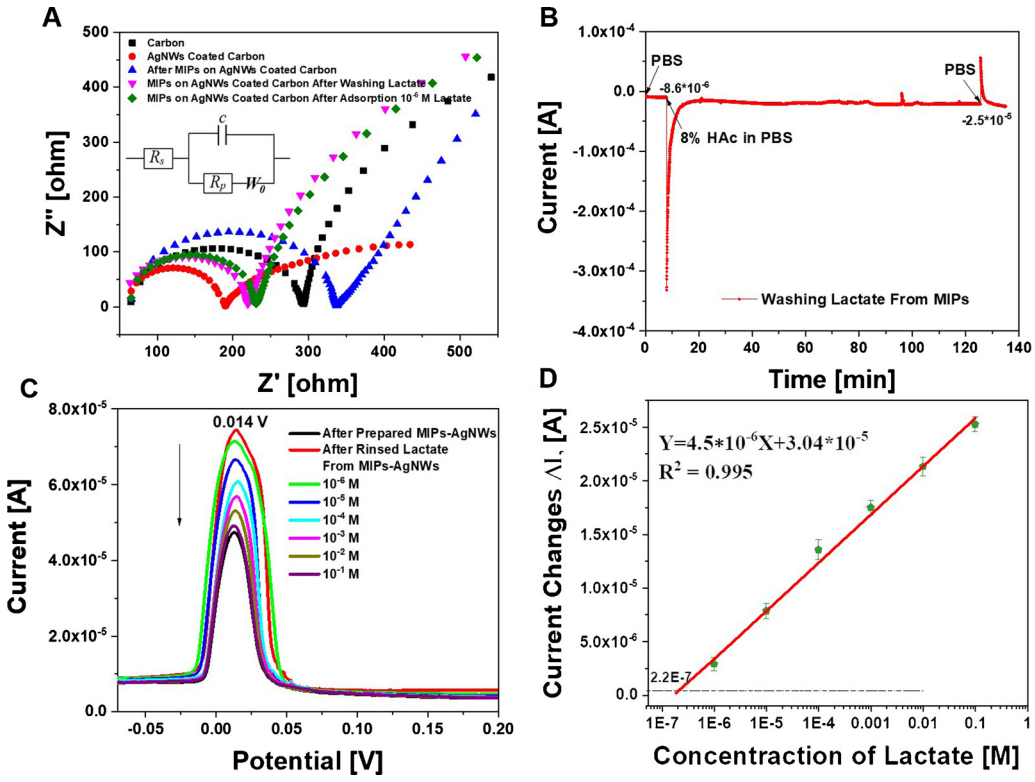



**Fig. 3.** (A) EIS measurements for the characterization of carbon WE (■), AgNWs coated carbon WE (●), MIPs-AgNWs coated carbon WE (▲), the MIPs-AgNWs coated carbon WE after removal of lactate (▼) and after adsorption of $10^{-6}$ M lactate for 20 min (◆), as measured in 5 mM $[Fe(CN)_6]^{3-/4-}$ and 1 M KCl mixture solution. (B) The I-T measurement for the washing dynamic of lactate molecules from MIPs-AgNWs with 8% acetic acid in PBS buffer for 2 h. Constant potential, 0.12 V; measurement intervals: 1 s. (C) DPV responses of the MIPs-AgNWs coated carbon electrode before and after removal of template molecules, and after incubated in different concentrations ($10^{-6}$ M to $10^{-1}$ M) of lactate in PBS solution for 20 min. (D) Calibration curve of the MIPs-AgNWs biosensor for the detection of lactate at the concentration from $10^{-6}$ M to 0.1 M in PBS.

### 3.3. EIS characterization of MIPs-AgNWs biosensor

EIS measurement was carried out for the investigation of the MIPs in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution (Fig. 3A). The semicircle of the Nyquist diagram for higher frequency corresponds to the electron transfer process. It shows the decrease of the semicircle after the carbon electrode was coated with Ag nanowires. The impedance increased after the polymerization of MIPs on AgNWs coated carbon electrode due to the non-conductive polymer hindered the electron transfer. In addition, after removal of the lactate molecules with 8% acetic acid in PBS solution (Fig. 3B), the impedance decreased with smaller semicircle on the EIS measurement. This should be ascribed to formation of recognition sites on MIPs-AgNWs, which improved the diffusion of $[Fe(CN)_6]^{3-/4-}$ and electron transfer. Finally, after specifically binding of lactate at the concentration of $10^{-6}$ M on the MIPs recognition sites, a slight increase of resistance was observed indicating successful bond of lactate to the MIPs.

The impedance spectrum of MIPs was successfully fitted based on the equivalent circuit with Zview software (Fig. 3A, inset). Here $R_s$ is the solution resistance, $C$ is double layer and the film capacitance, $R_p$ is the resistance of MIPs film, $W_O$ is the diffusion impedance with reflective boundary conditions [34]. After washing of lactate from MIPs, the resistance of MIPs was calculated as $R_p$ = 219.7 Ω, and the solution resistance $Rs$ = 105.5 Ω, which were comparable with the impedance evaluation based on the semicircle on EIS measurement.

### 3.4. Detection of lactate

The lactate level in human sweat has been reported up to 25 mM during strenuous exercise [36]. For the detection of lactate, the MIPs were rinsed firstly with 8% acetic acid in PBS for 2 h to remove the lactate molecules for the following adsorption of lactate (Fig. 3B). The I-T measurement showed the current intensity of MIPs increased from $-8.6 \times 10^{-6}$ A to $-2.5 \times 10^{-5}$ A after removal of template molecules. The current changes were comparable with the peak current changes ($\sim 2.7 \times 10^{-5}$ A) as measured by DPV (Fig. 3C). After removal of the template molecules, the sensor was incubated into lactate solution at concentrations from $10^{-6}$ M to 0.1 M for 20 min and a DPV voltammogram was obtained in PBS (Fig. 3C). The peak current intensity $I_a$ decreased with increasing the concentration of lactate. In Fig. 3D, the

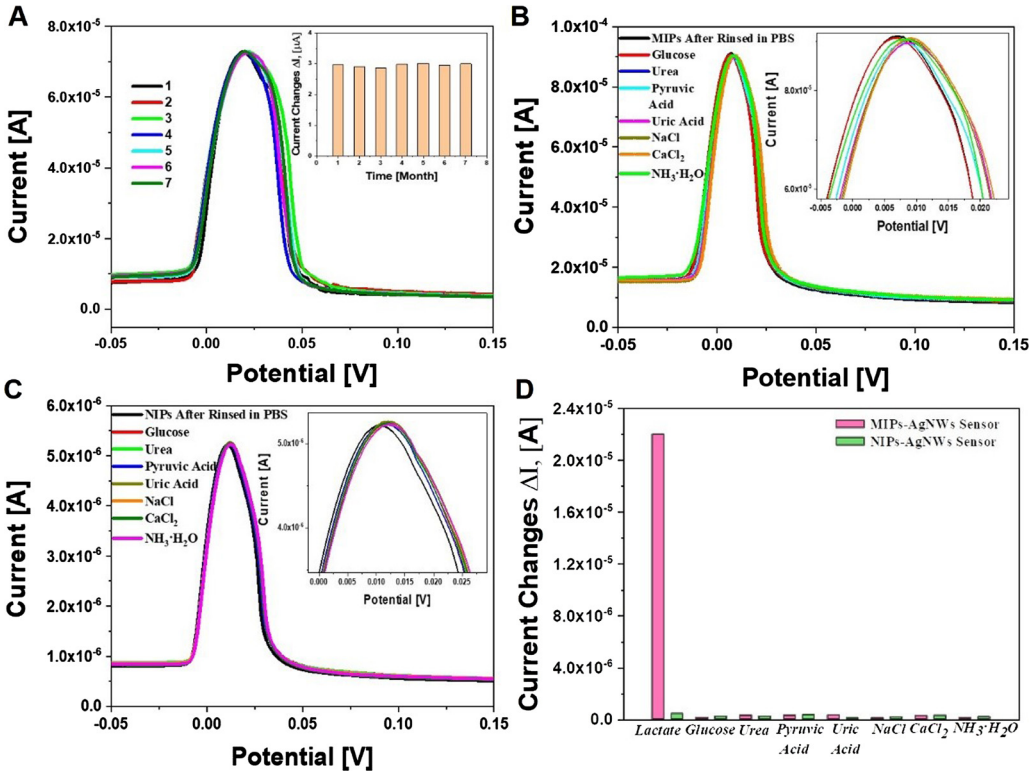



**Fig. 4.** (A) DPV responses for a MIPs-AgNWs sensor after incubation with $10^{-6}$ M lactate in PBS as measured every month during 7 months storage. The inset shows the peak current changes corresponding to the 7 measurements. (B) and (C) DPV responses for the MIPs-AgNWs and NIPs-AgNWs to the interference molecules (glucose, urea, pyruvic acid, uric acid, NaCl, $CaCl_2$, $NH_3{\cdot}H_2O$), respectively. (D) The sensor responses of MIPs-AgNWs and NIPs-AgNWs biosensor to the target molecule (lactate) and interference molecules (glucose, urea, pyruvic acid, uric acid, NaCl, $CaCl_2$, $NH_3{\cdot}H_2O$), respectively.

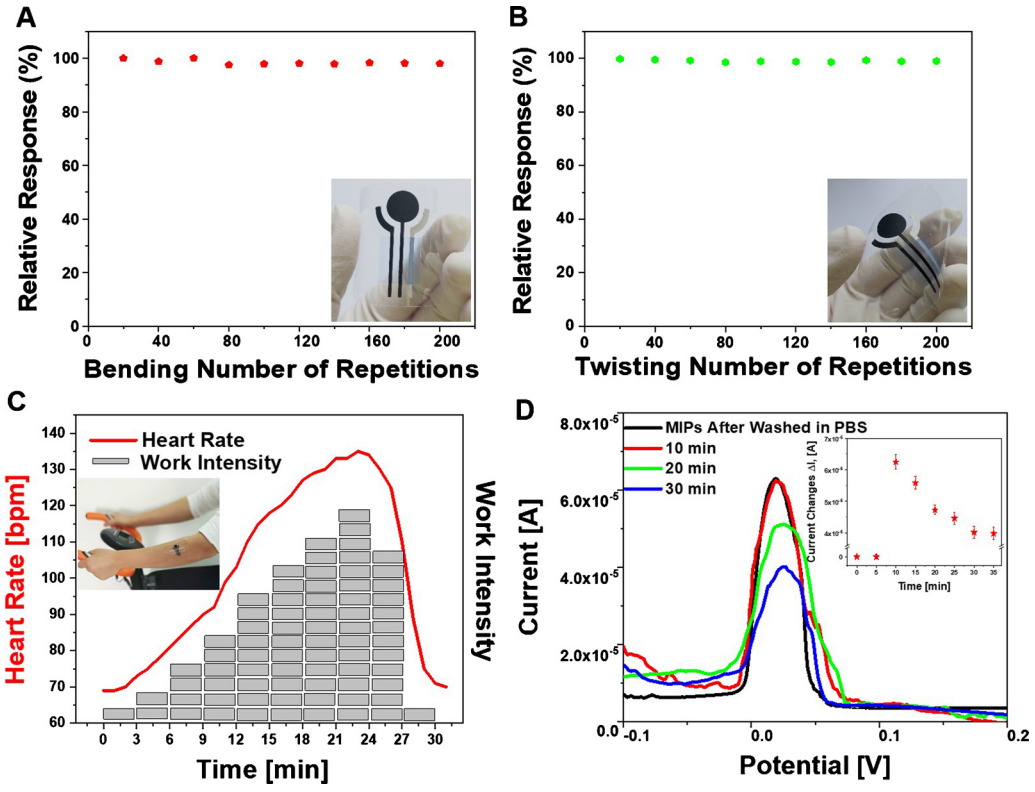



**Fig. 5.** The DPV relative response of MIPs-AgNWs biosensor undergoing repeated inward bending (A) and twisting (B) for 200 times, respectively. The DPV responses were performed in PBS from −0.2 V to 0.3 V. (C) The exercise intensity profile on a stationary cycle with a constant cycling rate of 3 min for a total of 30 min, and the corresponding heart rate of the volunteer during exercise. Inset: A MIPs-AgNWs biosensor was applied on a volunteer's arm. (D) The DPV of a MIPs-AgNWs biosensor as measured in PBS and on epidermis at 10 min, 20 min, and 30 min of cycling exercise. Inset: DPV peak current values of the MIPs-AgNWs biosensor were measured each 5 min during a 30 min of exercise. (For interpretation of the references to color in this figure citation, the reader is referred to the web version of this article).

calibration curve shows good linearity of the current intensity changes *versus* logarithm of lactate concentration from $10^{-6}$ M to 0.1 M, with the slope of $4.5 \times 10^{-6}$ (correlation coefficient of 0.995). The limit of detection (LOD) for the detection of lactate was estimated as low as 0.22 μM (S/N = 3) in the PBS.

To evaluate the stability and reproducibility of the biosensor, the MIPs-AgNWs chips were stored in an unsealed plastic box wrapped with tinfoil for 7 months at room temperature. The sensor chips were regenerated by rinsing with acetic acid and measured with DPV for the detection of lactate in PBS every month. The results showed the sensor responses ($\Delta I$) with variation recovery of 99.8% ± 1.7% for the detection of $10^{-6}$ M lactate during 7 months storage (Fig. 4A). Therefore, the

MIPs-AgNWs carbon electrode offered high reproducibility and stability for the further detection of lactate in the human sweat.

As we all know that, there are many kinds of metabolic substances in the human sweat, which may interfere with the detection of lactate. Therefore, the measurement of interference response on MIPs-AgNWs and NIPs-AgNWs biosensors were carried out at their physiological concentration in PBS. These interferents included glucose (0.17 mM), urea (10 mM), pyruvic acid (0.18 mM), uric acid (0.05 mM), NaCl (30 mM), $CaCl_2$ (5 mM) and $NH_3{\cdot}H_2O$ (5 mM), and the responses of the sensor to the interferents are negligible as shown in Fig. 4 (B–D). As compared with other interferents, urea, pyruvic acid and uric acid give the highest current changes around $3 \times 10^{-7}$ A, which is only 1.4% of the specific response for lactate (14 mM) binding ($2.2 \times 10^{-5}$ A). Similarly, the NIPs-AgNWs indicated negligible response which suggested its low nonspecific binding (Fig. 4C). Furthermore, the peak current signal increased after the addition of interferent may be ascribed to the increase of the ionic concentration in the solution. Accordingly, biosensor based on MIPs-AgNWs provided high stability and selectivity for the lactate detection. In addition, control experiment carried out on NIPs-AgNWs carbon electrode showed negligible binding with lactate molecules (Fig. 4D).

### 3.5. Flexible performance of MIPs-AgNWs biosensor

The sensor performance of MIPs-AgNWs was investigated after applied with mechanical force such as inward bending and twisting of 180 deg (Fig. 5A and B inset). The DPV curve was measured in PBS after every 20 times of bending or twisting. The relative response was calculated as the ratio the peak current change's ratio of $F_f = I_s/I_b$, where $I_b$ and $I_s$ are the peak current of the flexible electrode before and after applied with strains, respectively. As indicated in Fig. 5 (A–B), the relative responses of MIPs-AgNWs biosensor present recovery of 99.6% ± 0.9% and 99.2% ± 0.4% after being treated with bending and twisting for 200 times, respectively. Therefore, the MIPs-AgNWs biosensor showed high stability upon bending and twisting, which is beneficial for the following epidermal monitoring of lactate during exercise.

### 3.6. Epidermal sweat detection

The MIPs-AgNWs biosensors were applied on 6 volunteers' skin for the monitoring of lactate in the human sweat (Fig. 5C, inset). A thin-film PDMS (200 μm) with a sweat cell was used to attach the electrode biosensor to the volunteer's skin. The volunteers were asked to mount a cycle exercise as illustrated in Fig. 5C. The subject's heart rate was recorded during the cycling exercise as shown in the red line in Fig. 5C. The sweat lactate current changes were monitored by DPV responses every 5 min during the subject cycling. During the first 10 min of exercise, no DPV current response ($I = 0$) was observed due to the lack of perspiration present on the epidermis which resulted in open circuit of the three electrodes. After exercised for 10 min, the skin became moist on the biosensor surface obtained by naked eyes. As shown in Fig. 5D inset, the DPV peak current started to decrease after 10 min exercise due to the start of perspiration and the gradual buildup of lactate from the sweat. The typical DPV measurement was presented at 10 min, 20 min and 30 min of the exercise, respectively (Fig. 5D). The Fig. 5D inset also showed slow decrease of current signal at approximately 25 min, due to the decrease of exercise intensity. As expected, compared with calibration curve (Fig. 3D), the lactate concentration in sweat was estimated as 12.4 mM after the volunteer exercised for 30 min. In addition, this biosensor was applied for other 5 volunteers and appeared the similar current response. The results suggested the feasibility of the MIPs-AgNWs biosensor applied for the monitoring of sweat lactate level. Furthermore, there was no skin irritation and inflammatory thanks to the thin film of PDMS attached on the epidermis.

Overall, such an epidermal biosensor showed higher sensitivity for the evaluating of lactate in the perspiration, as compared with many of other lactate biosensors such as the non-enzymatic electrochemical lactate biosensor based on NiO and $Ni(OH)_2$ nanoparticles (LOD of $5.3 \times 10^{-4}$ M) [37], the MIPs/MWCNTs/PVC based biosensor (LOD of $7.3 \times 10^{-7}$ M) [38], enzymatic amperometric lactate sensor based on multiwalled carbon nanotubes (MWCNTs) and gold planar electrode (LOD of 0.96 μM) [39], enzymatic electrochemical tattoo biosensor for noninvasive lactate monitoring in human perspiration (detection range: 1 ~ 20 mM) [10]. Additionally, most of the current lactate biosensors still relied on the *in vitro* detection of perspiration lactate after the collection of sweat. However, our biosensor offered the feasibility for the on-body detection of perspiration lactate every 5 min during exercise, which provided the possibility for the real-time monitoring of human health condition based on their sweat.

## 4. Conclusions

We have developed a novel AgNWs based MIPs as an epidermal electrochemical biosensor for the non-invasive monitoring of sweat lactate on human's skin during exercise. The AgNWs network coated on the screen-printed carbon working electrode offered an excellent electric conductivity and stability. The lactate imprinted 3-APBA MIPs on AgNWs carbon electrode showed a high sensitivity and selectivity for the specific detection of lactate. The DPV measurement illustrated the lactate detection ranged from $10^{-6}$ M to 0.1 M with the LOD of 0.22 μM in PBS. Such AgNWs-MIPs biosensor maintained about 99.8% ± 1.7% of its sensitivity upon 7 months storage in the dark place at room temperature. Such a kind of epidermal biosensor provided a wonderful and sustainable method for non-invasive monitoring of other sweat metabolites, which would help for the monitoring of athlete and soldiers physiological conditions and the further application in the generalized healthcare domain.

## CRediT authorship contribution statement

**Qingwen Zhang:** Conceptualization, Methodology, Investigation, Writing - original draft. **Danfeng Jiang:** Conceptualization, Methodology, Writing - review & editing. **Changshun Xu:** Visualization, Investigation, Resources. **Yuancai Ge:** Methodology, Writing - review & editing. **Xiaohu Liu:** Methodology, Writing - review & editing. **Qingquan Wei:** Methodology, Writing - review & editing. **Liping Huang:** Visualization, Investigation, Resources. **Xueqian Ren:** Visualization, Investigation, Resources. **Chengde Wang:** Methodology, Resources, Writing - review & editing. **Yi Wang:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing - review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.snb.2020.128325.

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