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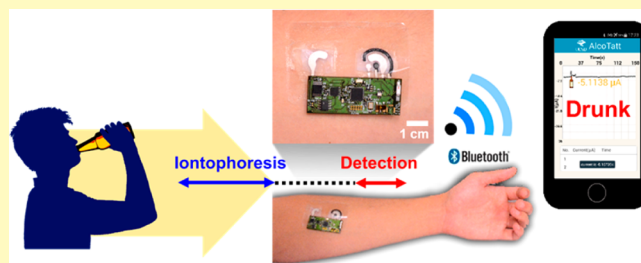
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S Supporting Information

ABSTRACT: In this paper we demonstrate a wearable tattoo-based alcohol biosensing system for noninvasive alcohol monitoring in induced sweat. The skin-worn alcohol monitoring platform integrates an iontophoretic-biosensing temporary tattoo system along with flexible wireless electronics. The wearable prototype enables the transdermal delivery of the pilocarpine drug to induce sweat via iontophoresis and amperometric detection of ethanol in the generated sweat using the alcohol-oxidase enzyme and the Prussian Blue electrode transducer. The new skin-compliant biosensor displays a highly selective and sensitive response to ethanol. On-body results with human subjects show distinct differences in the current response before and after alcohol consumption, reflecting the increase of ethanol levels. The skin-worn alcohol sensor is coupled with a flexible electronics board, which controls the iontophoresis/amperometry operation and transmits data wirelessly in real time via Bluetooth communication. The new wireless epidermal iontophoretic-biosensing system offers considerable promise for noninvasive monitoring of alcohol consumption in practical settings and can be readily expanded toward the monitoring of additional analytes.

KEYWORDS: sweat alcohol, tattoo sensor, wearable, wireless electronics, iontophoresis



Unsafe levels of alcohol consumption can lead to vehicle crashes, violence, and the degenerated health of heavy drinkers.^{1,2} Such alcohol-related incidents and health concerns continue to rise rapidly across the globe, leading to considerable socioeconomic costs. Accordingly, there are tremendous needs for an accurate easy-to-use alcohol measuring device for use by law enforcement personnel, service/hospitality industry, or individual drinkers to provide a convenient means to monitor alcohol consumption. Although different methods have been used for determining alcohol consumption, including direct measurements of urine, blood, saliva, breath, or sweat, or verbal/interview measures, Blood Alcohol Concentration (BAC) is the most commonly used indicator of alcohol intoxication.^{3–6} Unfortunately, blood samples cannot currently be obtained without penetrating skin, typically via a lancet pricking a finger or earlobe, which can be painful and inconvenient, and demands user compliance. Such blood sampling methods limit the general use of alcohol monitoring devices to the most extreme cases (e.g., law enforcement), rather than as a general-purpose tool that can monitor a user's alcohol levels and warn or prevent what could amount to a catastrophe. Accordingly, there are considerable demands for developing an alternative approach for measuring BAC indirectly in a noninvasive and real-time manner.

Currently, breathalyzers are the most commonly used devices to indirectly estimate BAC, and operate by measuring breath

alcohol concentration (BrAC). BrAC instruments calculate BAC by following Henry's law, but may compromise accuracy since the results can be easily affected by humidity, temperature, and individual traits.^{7,8} BrAC can also generate false alarms due to alcohol vapor associated with consumer products (e.g., mouthwash and breath freshener), and environmental factors (e.g., paint fume, varnish, and chemicals).⁹ BAC can also be estimated by measuring transdermal alcohol concentration (TAC), because a person's perspiration can contain traces of alcohol after alcohol consumption.¹⁰ Two wearable transdermal alcohol sensor devices (SCRAM, Giner WristAS) have been developed for detecting local ethanol vapor concentration in insensible perspiration over the skin, which is not secreted by sweat glands.^{11,12} However, these devices suffer from long time delays (0.5–2 h) compared to BAC estimated by breathalyzers.¹¹ Therefore, the development of a new prototype to monitor BAC noninvasively in real time is highly desired.

Recent work¹³ has combined iontophoresis and biosensing processes for measuring alcohol level in induced sweat (sensible sweat) based on the established correlation between the two fluids during alcohol consumption.^{14,15} Such an operation showed that the maximum ethanol level in both fluids, blood

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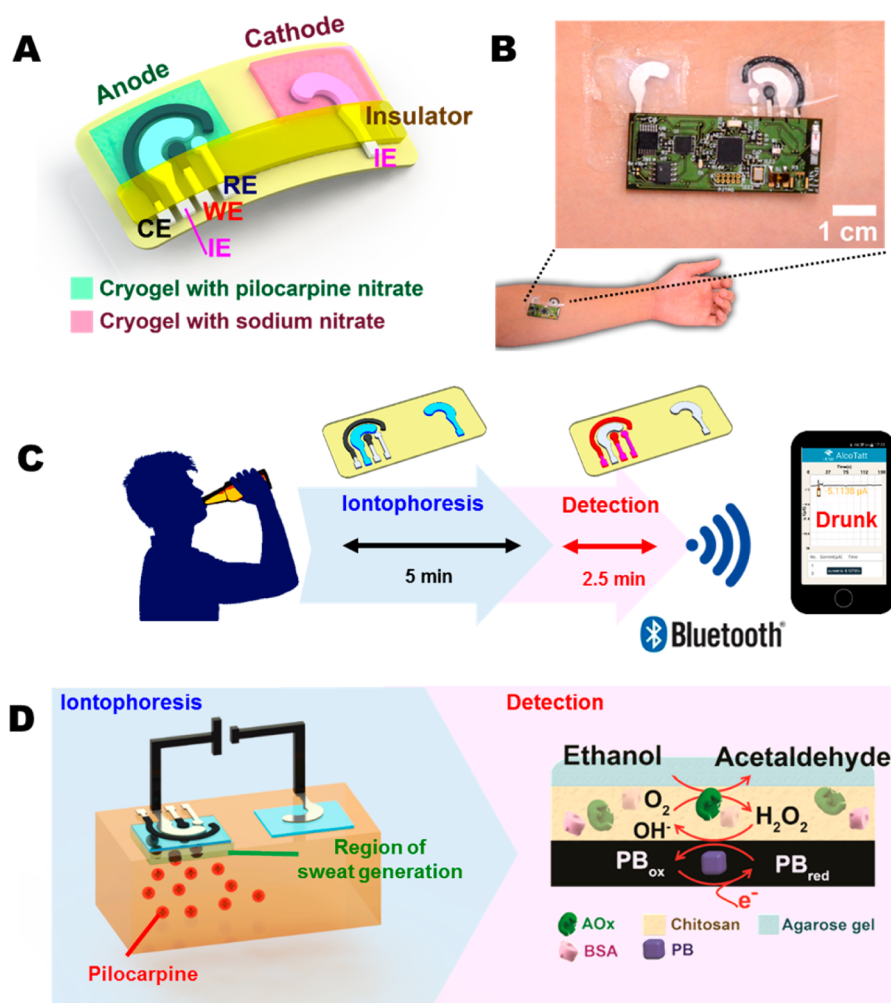


Figure 1. Tattoo-based transdermal alcohol sensor. (A) Schematic diagram of an iontophoretic-sensing tattoo device, containing the iontophoretic electrodes (IEs; anode and cathode) and the three sensing electrodes (working, reference, and counter electrodes: WE, RE, and CE, respectively). (B) Photograph of an alcohol iontophoretic-sensing tattoo device with integrated flexible electronics applied to a human subject. (C) Schematic diagram of a wireless operation of the iontophoretic-sensing tattoo device for transdermal alcohol sensing. In the diagrams of the tattoo-base device, blue and red highlights show the active zones during iontophoresis and amperometric detection, respectively. (D) Schematic diagram of constituents in the iontophoretic system (left) and of the reagent layer and processes involved in the amperometric sensing of ethanol on the working electrode (right).

and collected sweat induced by pilocarpine iontophoresis, reached at nearly the same time, compared to the time delays of common TAC sweat sensors using insensible sweat (Giner WristAS). However, the electrochemical sensing system was combined with a bulky nonwearable iontophoretic system, and required replacement of electrodes between the iontophoresis and amperometric detection steps.

Here we demonstrate a wearable temporary-tattoo biosensing system capable of real-time noninvasive alcohol monitoring via integration of printed and flexible iontophoretic-sensing electrodes with wireless electronics (Figure 1). Our study is built upon recently developed noninvasive wearable chemical sensors for monitoring chemical markers in sweat toward health or fitness applications.^{16–22} Our laboratory has developed body-compliant tattoo-based epidermal electrochemical sensors for noninvasive monitoring of sweat electrolytes, metabolites, or trace metals.^{23–28} Recently, we reported a tattoo-based noninvasive glucose monitoring system that combines reverse-iontophoresis with an amperometric enzyme electrode.²⁵ Gonzalo-Ruiz et al.²⁹ also demonstrated the coupling of a potentiometric ion selective electrode with iontophoresis on a

screen-printed electrode to monitor chloride ion in sweat for early determination of cystic fibrosis.²⁹

In the following sections we describe an integrated tattoo-based wearable system for effective noninvasive ethanol monitoring based on coupling sweat-inducing iontophoresis and amperometric enzymatic biosensing (Figure 1A), along with a flexible supporting electronic readout module featuring wireless telemetry (Figure 1B). The new skin-worn low-cost noninvasive alcohol monitoring device enables real-time alcohol measurements in induced sweat and obviates the need for lengthy and costly procedures. This represents the first example of integrating a drug-loaded iontophoretic operation and electrochemical amperometric biosensing of metabolite on a wearable tattoo platform, in general, and for tattoo-based flexible epidermal alcohol sensor system, in particular.

The new wearable epidermal alcohol sensor system uses constant-current iontophoresis for inducing sweat by delivering the drug pilocarpine across the skin, followed by amperometric biosensing of the sweat ethanol (Figure 1C). The latter relies on an alcohol-oxidase (AOx) enzymatic electrode along with a printed Prussian Blue (PB) electrode transducer. Such

integration of the iontophoretic and amperometric operations onto a single flexible tattoo platform obviates the need for replacing the electrodes between iontophoresis and detection steps as in prior work.¹³ This coupling has been accomplished by adding a pair of conductive Ag/AgCl iontophoretic electrodes to the three-electrode amperometric sensing system using a specific design pattern essential for integrating the two systems (Figure 1A). All the electrodes were fabricated by a screen-printing technique on the wearable temporary-tattoo paper to offer cost-effective mass production along with convenient placement and removal from the skin. For realization of real-time alcohol monitoring with the wearable sensor, the tattoo alcohol sensor was integrated with flexible printed electronic circuitry (Figure 1B), which controls the entire iontophoretic-sensing operation wirelessly and transmits the data to laptop or mobile devices via Bluetooth communication (Figure 1C). Both the skin-worn sensor and electronic board are flexible and compatible with the non-planarity of the epidermis and offer resistance to mechanical stress from the wearer's movement.

The performance of the developed sensor was evaluated first in a buffer medium for its sensitivity, selectivity, and potential cross talk between the iontophoretic and sensing operations. Subsequently, the on-body iontophoresis operation was optimized on the human skin and the epidermal sensor was tested with human subjects under ingestion of alcoholic beverages. The on-body results (including corresponding control experiments) indicate that the new wearable epidermal alcohol sensor system holds considerable promise for reliable decentralized noninvasive alcohol monitoring in diverse practical settings toward saving lives and supporting the criminal justice system.

■ EXPERIMENTAL SECTION

Chemicals and Instruments. Alcohol oxidase (AOx, from *Pichia pastoris*, 10–40 units/mg protein), chitosan, bovine serum albumin (BSA), potassium phosphate monobasic (K_2PO_4), potassium phosphate dibasic (K_2HPO_4), ethanol, acetic acid, pilocarpine nitrate, sodium nitrate, L(+)-ascorbic acid, uric acid, agarose type IV, and poly(vinyl alcohol) (PVA, average molecular weight = 70 000–100 000) were purchased from Sigma-Aldrich (St. Louis, MO). All reagents were used without further purification. A μ Autolab type III PGSTAT302N (Metrohm), controlled by Autolab NOVA software v 1.11.2, was applied for the electrochemical characterization in buffer medium and in on-body evaluation.

Fabrication, Chemical Modification, and Transfer Process of a Tattoo Sensor. Patterns for printing the sensor were designed in AutoCAD (Autodesk, San Rafael, CA) and transferred to stainless steel plates (12×12 in.²) etched to fabricate stencils (Metal Etch Services, San Marcos, CA). Temporary transfer tattoo paper kits were obtained from HPS Papilio (Rhome, TX). The silver/silver chloride (Ag/AgCl) ink (E2141 Ercon Inc., Wareham, MA) and PB conductive carbon (C2070424P2, Gwent Group, UK) were screen-printed on the substrate by using an MPM-SPM semiautomatic screen-printer (Speedline Technologies, Franklin, MA). As illustrated in Figure 1A, the tattoo sensor design consists of iontophoresis and pseudo reference electrodes patterned from Ag/AgCl ink, and the working and counter electrodes patterned using the PB conductive carbon ink. The diameter of the printed working electrode was 3 mm. A transparent insulator was screen-printed over the surface of the electrode pattern to confine the electrodes and contact areas. The Ag/AgCl ink was cured at 90 °C for 10 min, while the PB conductive carbon ink was cured at 80 °C for 10 min in a convection oven.

In order to prepare the alcohol biosensor, the printed working electrode was functionalized with an enzymatic layer. The AOx enzyme, BSA stabilizer, and chitosan solution (0.5 wt % in acetic acid

solution) were mixed together in a ratio of 8:1:1 (v/v/v). Afterward, a 4 μ L droplet of the mixed solution was casted on the electrode. After air-drying, the electrode was covered with a 2 μ L droplet of chitosan solution (0.5 wt %). Then, the electrode was dried under ambient conditions. The agarose hydrogel was prepared by heating a continuously stirred agarose solution (2% w/v) with 0.1 M potassium phosphate buffer (pH 7.0) until its complete dissolution. The dissolved agarose hydrogel was then casted on the working enzyme electrode. Subsequently, the prepared cryogels (2.0×1.5 cm²) were soaked in 1% pilocarpine nitrate and 1% sodium nitrate (for 1 h) and then covered on the anode and cathode compartments, respectively. The cryogels were made as following procedure by freezing and thawing sequences of PVA solution. First, a 5.0% w/v PVA solution was prepared in deionized water by heating the solution to 120 °C and then cooled down to room temperature. After the mixture was cooled down, it was placed in the ice bath and the pH was adjusted to pH 1.0 by adding 5 M hydrochloric acid. Subsequently, the cross-linker glutaraldehyde was added to give a final concentration 0.5% w/v. The final mixture was then stirred for 1 min and poured onto the glass mold to set in –20 °C freezer overnight.

Evaluation of Sensor Performance in Buffer Medium. The electrochemical performance of the alcohol tattoo biosensor was evaluated first in vitro in a 0.1 M phosphate buffer (pH 7.0) solution (PBS). The chronoamperometric response was measured, after 1 min immersion in the test solution, by stepping the potential to –0.2 V (vs Ag/AgCl) for 60 s. Calibration plots were obtained using 3 mM increments of the ethanol concentration, up to 36 mM in the buffer solution. The selectivity was examined by measuring the response to 10 mM ethanol in the presence of relevant electroactive species: 0.2 mM glucose, 10 mM lactate, 84 μ M creatine, 10 μ M ascorbic acid, and 60 μ M uric acid.³⁰ The influence of pilocarpine upon the activity of the PB transducer was examined using cyclic voltammetry (CV) of the bare PB electrode; the CV was recorded in PBS solution containing 1% pilocarpine solution (dissolved in PBS). To address the pilocarpine interference, the working enzyme electrode was modified by drop-casting 2% agarose gel containing PBS. The recovered PB activity with agarose gel modification was confirmed by CV in 1% pilocarpine solution (dissolved in DI water) in comparison with a bare PB electrode (without the agarose gel modification).

The resilience of the tattoo biosensor to mechanical strain was examined by measuring the ethanol response after repeated 90° bending. The tattoo sensors were transferred to transparent plastic substrate to mimic the flexible properties of the skin. The amperometric response was recorded every 20 times of repeated bending up to 120 times.

On-Body Evaluation of a Tattoo Alcohol Biosensor. The epidermal evaluation on human subjects was conducted in strict compliance following a protocol approved by the institutional review board (IRB) at the University of California, San Diego. A total of nine healthy volunteers were recruited for on-body evaluation of the developed sensor before and after consumption of alcoholic beverages. In all experiments, the tattoo biosensors were placed on the subjects' arms, and ethanol sweat measurement was performed using the iontophoresis-amperometry operation to obtain the current response at BAC 0.000% ("before drinking"). During the iontophoresis process, a constant current of 0.6 mA (0.2 mA cm^{–2}) was applied for 5 min through a cryogel layer between the two iontophoresis (i.e., anode and cathode) electrodes to deliver pilocarpine and induce sweat. The applied current density and duration were optimized and selected using on-body test as a trade-off between efficient sweat generation and the subject's compliant. For example, high currents that result in efficient pilocarpine drug delivery, also lead to skin burning/irritation. The applied time (5 min) was also selected based on the on-body results; 5 min is minimum duration to induce sweat and detect the alcohol sweat current signal. This was evaluated by multiple subjects ($n = 9$), since skin permeability varies among individuals. It was followed by a 5 min rest period during which the sweat was generated. Subsequently, the amperometric response of ethanol in sweat (BAC 0.000%) was recorded at an applied potential of –0.2 V (vs Ag/AgCl) for 150 s with a benchtop potentiostat. The subject then consumed an

alcoholic beverage (12 oz. of beer or 5 oz. of table wine) for 5 min and waited for 10 min to allow alcohol diffusion in blood. Upon consumption, the alcohol passes through the stomach and gastrointestinal (GI) tract into the bloodstream. Afterward, it diffuses to the surrounding body tissues, including the skin. The iontophoresis/detection cycle was then repeated to measure the corresponding ethanol sweat concentration.

Along with the above on-body iontophoresis-sensing experiments involving alcohol consumption, three different control experiments (without drinking, without enzyme modification, and without iontophoresis) were performed. The sensor response toward sweat ethanol was confirmed first in comparison with measurements without drinking alcoholic beverages. The selectivity of the sensor toward ethanol was evaluated also by comparison with the response of the enzyme-free electrodes. The effect of iontophoresis was verified by control experiments without the iontophoresis step. These three different control experiments were carried out under otherwise identical conditions, ensuring that the observed response is solely due to increased alcohol level following drinking. Each set of measurement cycles was accompanied by simultaneous BAC measurements using a commercial FDA-approved breathalyzer (Alcovisor Mars Breathalyzer, Hong Kong) to validate the sensor performance.

Several additional on-body tests involved estimating the correlation between BAC and the current response of the tattoo sensor with repeated consumption of wine. These involved the same iontophoresis-amperometry experimental procedure using two sets of repeated drinking and measurement. The current response was measured initially before drinking (when BAC is 0.000%) using the iontophoresis-sensing operation. Then, the current response of such operation was recorded following the first and second drinking.

Flexible Wireless Instrumentation Electronics. The flexible tattoo-based iontophoretic alcohol monitoring patch must connect to instrumentation electronics to control the iontophoretic and amperometric electrodes and readout data. While initial testing and characterization of the biosensor system have been carried out using benchtop equipment (CH Instruments), such a bulky electrochemical analyzer limits the system-level attractiveness of having a thin temporary-tattoo design. Accordingly, the tattoo sensor was connected directly to a flexible printed circuit board (PCB) containing commercial off-the-shelf (COTS) integrated circuits for instrumentation, control, and telemetry in a thin, wearable form-factor. Specifically, the PCB employs a Texas Instrument (TI) CC2541 Bluetooth Low Energy (BLE) System-on-Chip for communication and processing. Iontophoretic current injection is achieved via a TI LM334 current source, which applies a 0.6 mA current between the iontophoretic cathode and anode electrodes. After 5 min of current injection, the LM334 was disconnected by control signals from the CC2541. Following an additional 5 min, a TI LMP91000 chemical sensing analog front end was enabled, and applied a -0.2 V potential across the amperometric electrodes for constant-potential amperometry experiments. The potential was held for up to 60 s, or until the resulting amperometric current, also read by the LMP91000, stabilized. The resulting current throughout the 60 s experiment was sampled and digitized by an on-board analog-to-digital converter (ADC) on the CC2541, and was transmitted via a Johanson Technology 2.45 GHz chip antenna (2450AT42A100) and impedance matched balun (2450BM15A0002) in a 2-byte format to a Bluetooth 4.0-enabled receiver. The receiver decoded the data and presented the results in a Python-based graphical interface on a desktop or laptop. The flexible PCB was powered by two 396/397 watch batteries (2×1.55 V, 33 mAh each) mounted in series and conditioned via a TPS61220 boost converter and an LM4120 low-dropout voltage regulator. The PCB prototype, shown in Figure 1B, measured 2 cm \times 5 cm.

A stainless steel sheet was cut in 2 mm \times 10 mm size and attached on connection point of electrodes using conductive silver epoxy adhesive for detachable magnetic connection with magnets soldered on flexible board. The sensor-board assembly is shown in Figure 1B; on-body evaluation proceeded following the same protocol described in the previous section. The resulting current response was transmitted to a laptop via a Bluetooth 4.0 and was displayed using a custom-made

graphical interface. The integration of the wearable tattoo sensor with wireless electronics and its operation are shown in the Supporting Information.

RESULTS AND DISCUSSION

Rationale for an Iontophoretic-Biosensing System of a Tattoo-Based Alcohol Sensor. A novel aspect of the new wearable alcohol biosensor is the integration of pilocarpine-iontophoretic and amperometric detection systems and their coupling onto a single flexible tattoo-based device. Combining both systems and operations into a single skin-worn platform requires a specific electrode design. The alcohol tattoo sensor system thus consisted of the iontophoretic electrodes (anode and cathode) and the three amperometric sensing electrodes (WE, RE, and CE) located in the anode compartment (Figure 1A). The pair of iontophoretic electrodes is responsible for inducing sweat by delivering the pilocarpine drug from the anode compartment. These sweat-generating electrodes are thus positioned in the middle of sensing electrodes. Such a specific electrode layout is essential for an optimal integration of iontophoretic and detection systems and its skin-worn operation. The screen-printed three-electrode detection system consisted of the PB-based working electrode, the pseudo Ag/AgCl reference electrode, and the PB conductive carbon counter electrode. Adding the third counter electrode to our earlier two-electrode tattoo biosensor design²⁵ offers improved potentiostatic control. In addition, in order to achieve the fully integrated system, with simultaneous iontophoresis, enzymatic reaction, and amperometric detection, the position of the iontophoresis electrode and its distance from sensing electrodes are crucial factors considering the small volume of generated sweat. The iontophoresis protocol was optimized in preliminary experiments for effective sweat generation avoiding skin irritation. Cryogel layers covered all the iontophoretic electrodes for ensuring proper contact with the skin, which was soaked with 1% pilocarpine nitrate and sodium nitrate on the anode and cathode, respectively, to deliver these species across the skin by applying a mild electrical current. Cryogel has been selected as the drug sorbent based on our preliminary experiments (compared with filter paper and agarose gel), because it offers a porous sponge structure, elasticity, and biocompatibility. These properties were obtained from ice porogen of PVA gel (i.e., ice crystals acting as highly porous molds under subzero temperature; they are melted away after the gel has set) which allows high loading of pilocarpine and good permeation of sweat across the gel.³¹ During the iontophoresis operation, the cationic pilocarpine drug was delivered across the skin at the anode electrode (Figure 1D).³² As expected, sweat was generated at the pilocarpine-loaded iontophoresis electrode a short time after delivering the drug, causing no apparent skin irritation and burning.

The amperometric biosensing of alcohol in the generated sweat relied on an AOx-coated screen-printed PB transducer that offers specific low-potential electrocatalytic detection of the hydrogen peroxide product of the AOx enzymatic reaction.³³ The AOx was immobilized on the working electrode by drop-casting with BSA and chitosan, and then covered with agarose gel containing PBS (K^+) to provide consistent and stable electrolyte level essential for electron-shuttling activity of PB (Figure 1D) and the electrochemical detection. The resulting effect of the agarose gel in the iontophoretic-sensing system is discussed and confirmed experimentally in following sections.

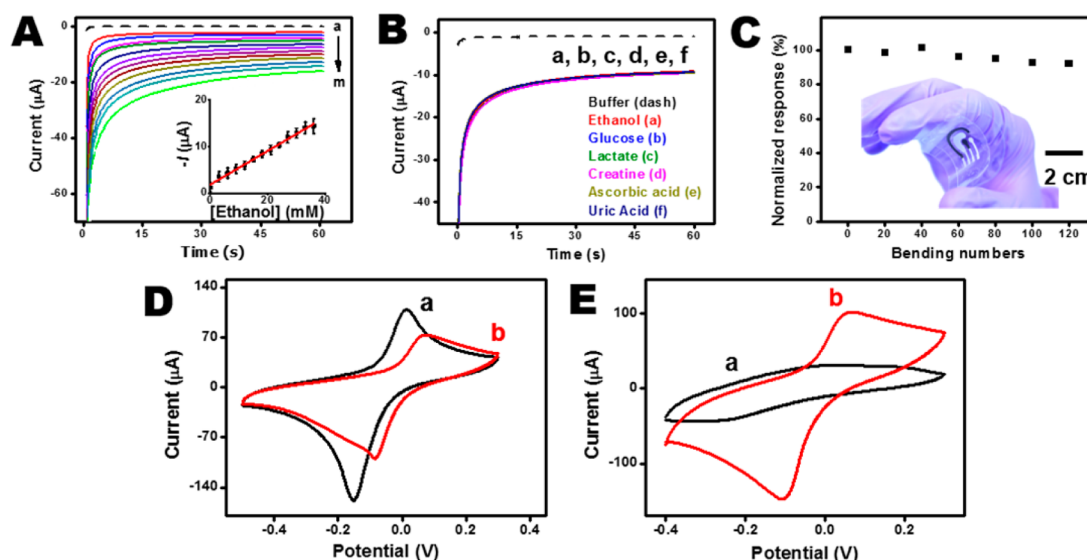


Figure 2. (A) Amperometric response of the tattoo-based alcohol sensor to increasing ethanol concentrations from 0 mM (a) to 36 mM (m) in pH 7 buffer solution with 3 mM increments. The inset shows the corresponding calibration plot. Error bars represent the standard deviations ($n = 3$). Potential step to -0.2 V (vs Ag/AgCl). (B) Interference study in the presence of 10 mM ethanol (a), followed by additions of 0.2 mM glucose (b), 10 mM lactate (c), 84 μ M creatine (d), 10 μ M ascorbic acid (e), and 60 μ M uric acid (e); potential step to -0.2 V (vs Ag/AgCl); medium, pH 7 phosphate buffer solution. (C) Mechanical deformation tests of the alcohol tattoo sensor involving 90° bending. Relative amperometric current response to 10 mM ethanol during a series of 120 deformations, recorded after 20 such repeated bending tests in pH 7 buffer solution. Photograph of the 90° bending of the transferred tattoo electrode on a transparent plastic substrate. Potential step to -0.2 V (vs Ag/AgCl). (D) Cyclic voltammograms of the tattoo-based alcohol sensor in pH 7 buffer solution (a) and 1% pilocarpine dissolved pH 7 buffer solution (b). (E) Cyclic voltammograms of the tattoo-based bare Prussian Blue (PB) electrode (a) and at the PB electrode modified with 2% agarose gel (b) recorded in an in 1% pilocarpine solution (dissolved in DI water).

Characterization of an Alcohol Tattoo Sensor in Buffer Medium. The electrochemical performance of the alcohol biosensor was validated first in buffer medium over a wide dynamic concentration range of 0–36 mM ethanol, corresponding to the physiological level of ethanol in sweat. Figure 2A displays well-defined chronoamperometric response to 12 successive increments of 3 mM ethanol in a potassium phosphate buffer solution (b–m). These additions result in well-defined amperometric response over the entire ethanol range, with convenient quantitation (stable current) observed ~ 30 s after the potential step. The corresponding calibration plot, shown in the inset of Figure 2A, is highly linear over the entire range examined (slope, $0.362 \pm 0.009 \mu\text{A}/\text{mM}$; intercept, $1.810 \pm 0.170 \mu\text{A}$; correlation coefficient, $R^2 = 0.993$; $n = 3$).

Since human sweat contains various physiologically relevant interferences (e.g., glucose, uric acid, lactate, ascorbic acid, and creatine), it is important to examine the selectivity of the new biosensor toward the target ethanol. Figure 2B evaluates the influence of several potential interferences upon the response to 10 mM ethanol. These data demonstrate that the various coexisting compounds have a negligible effect upon the ethanol response (a vs b–f). Such high selectivity reflects coupling of the specific enzymatic reaction with the extremely low detection potential of the peroxide product at the PB transducer.

Wearable applications of the tattoo alcohol biosensor require resilience against mechanical stress. The endurance of tattoo alcohol sensor against mechanical deformations was evaluated by measuring its performance under repeated 90° bending. The tattoo-based biosensor underwent 120 times of repeated bending and their amperometric response was measured after every 20 times of bending. As illustrated in Figure 2C, the current response to 10 mM ethanol was retained after such repeated bending, indicating the robustness of the tattoo

electrodes against mechanical deformations expected in practical wearing situations.

The new printable electrode system represents the first attempt to combine iontophoresis and detection operations on a single skin-worn tattoo platform. Therefore, potential cross talk between the iontophoresis and detection electrodes should be critically assessed before integration of the two systems. Accordingly, we examined the effect of pilocarpine upon the PB activity (Figure 2D). Pilocarpine is a large positively charged molecule, and the electron shuttling activity of PB can be influenced by pilocarpine due to the vulnerability of PB against other positive ions in electrolytes.³² Pilocarpine can thus decrease the electron shuttling ability of PB, as indicated from the CVs of Figure 2D. These data illustrate 39.7% diminished redox peak currents of PB in phosphate buffer medium in the presence of pilocarpine, along with slight shifts in the peak potentials (a vs b).

In order to prevent interference of pilocarpine upon the electron shuttling activity of PB, the enzyme-coated PB working electrode was covered with a 2% agarose gel. The agarose gel layer containing buffer electrolytes provides sufficient potassium ions to address and “buffer” the pilocarpine effect on the PB electrode and fully restore its electron shuttling ability. Figure 2E illustrates the recovered CV redox peaks in 1% pilocarpine solution (dissolved in DI water) following modification of the enzyme-modified PB working electrode with a 2% agarose gel. While no PB peaks are observed at the unmodified electrode immersed in the 1% pilocarpine solution (dissolved in DI water) due to the absence of electrolytes (a), the PB redox signals can be observed at the electrolyte-containing agarose modified electrode (b). It indicates that the agarose gel layer eliminates the pilocarpine interference by impeding diffusion of pilocarpine to working electrode and

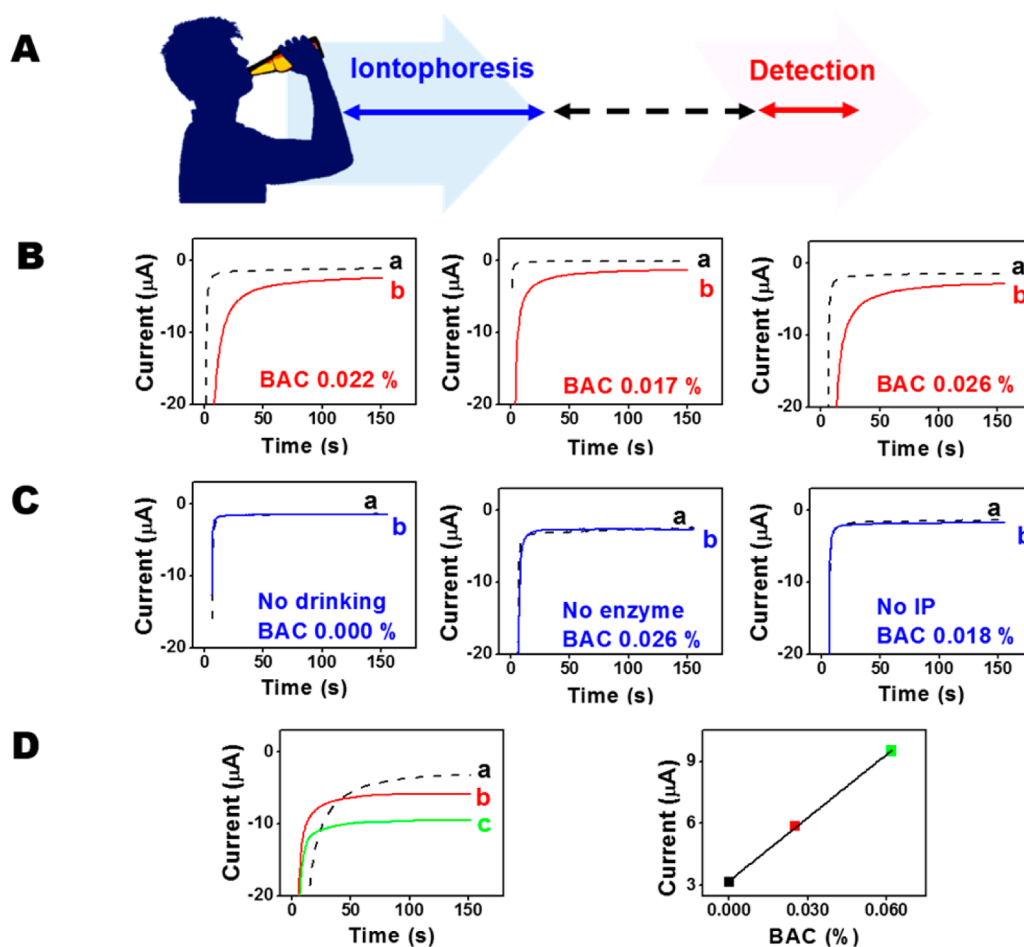


Figure 3. (A) Schematic procedures of on-body study of alcohol sensing. Chronoamperograms obtained for noninvasive alcohol detection obtained from human subjects wearing the alcohol tattoo sensors and operated by the benchtop potentiostat; (B) experiments with consumption of 12 oz of beer measured from three different human subjects before (plot “a”) and after drinking alcohol beverage (plot “b”); (C) control experiments without drinking (left: first measurement, plot “a”; second measurement, plot “b”), without enzyme immobilization (middle: before drinking, plot “a”; after drinking, plot “b”), and without iontophoresis (IP) (right: before drinking, plot “a”; after drinking, plot “b”); (D) experiments to demonstrate correlation between BAC level and current response from the tattoo biosensor measured. The left plot shows chronoamperograms obtained before drinking (a, BAC: 0%), after drinking 5 oz of wine (b, BAC: 0.025%) and 10 oz of wine (c, BAC: 0.062%). The right plot shows the correlation between current response obtained from the tattoo sensor and BAC level. The BAC level was obtained by breathalyzer. Potential step to -0.2 V (vs Ag/AgCl).

providing additional electrolytes to facilitate the redox function of PB.

On-Body Alcohol Monitoring on Human Subjects.

After evaluation of the tattoo sensor performance *in vitro*, we tested the on-body operation of the skin-worn alcohol tattoo sensor with human subjects. First, the detection ability of the tattoo alcohol biosensor was confirmed under consumption of alcoholic beverage. Figure 3B displays on-body amperometric results obtained by three different subjects before and after alcohol consumption, using the protocol described in the Experimental Section. A distinct increase of the current response is observed by comparing the signals from these three subjects. A second amperometric response (after drinking) was measured 20 min after alcohol consumption, along with a parallel BAC measurement. Even though three subjects consumed the same amount of alcohol, their BAC values—measured by breathalyzer—vary due to their different alcohol metabolism rates and body weights. For example, three subjects in Figure 3B have 0.022%, 0.017%, and 0.026% BAC, respectively, after consuming the same amount of alcohol. The

alcohol sweat concentration can be estimated from BAC through the following equation:¹³

$$\text{BAC (g L}^{-1}\text{)} = 0.71 \times \text{sweat ethanol concentration (g L}^{-1}\text{)}$$

$$r = 0.912$$

The BAC of the above subjects can thus be converted to 6.7 mM, 5.2 mM, and 7.9 mM alcohol sweat concentrations, respectively. Note also the different current signals for subjects with the same BAC values that reflect their different skin permeabilities and sweat compositions. None of the subjects reported perceptible discomfort during these on-body measurements. Three control experiments (no drinking, no enzyme modification, and no iontophoresis) were performed to verify the absence of potential false alarms from the on-body results obtained under alcohol consumption. None of the control experiments showed a current difference (Figure 3C). For example, the control experiment without enzyme modification (Figure 3C, No enzyme modification) clearly demonstrates the high specificity of the tattoo sensor toward sweat alcohol. Similarly, the control experiment without drinking (Figure 3C,

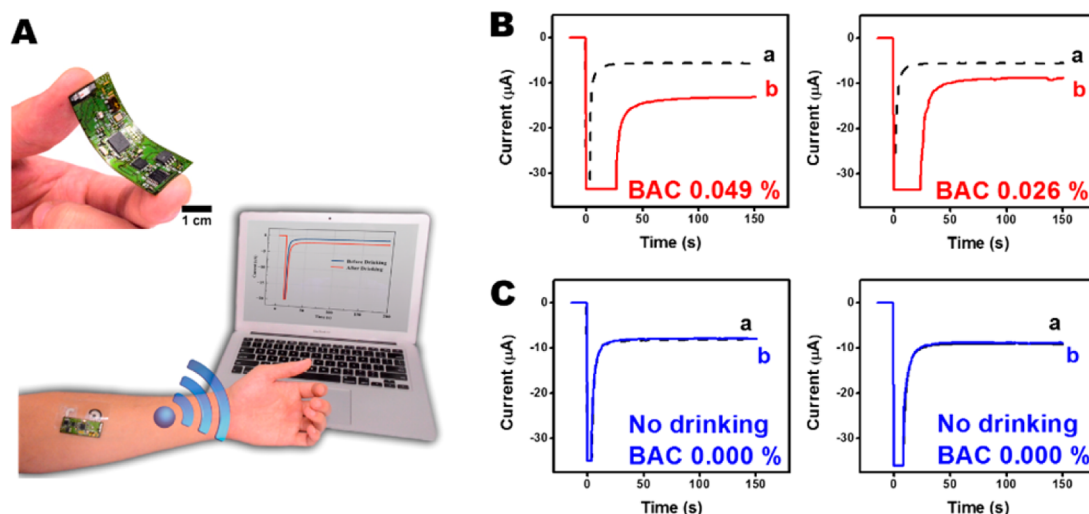


Figure 4. (A) Photograph of an alcohol iontophoretic-sensing tattoo device with an integrated flexible electronics applied to a human subject and the wireless communication with a laptop computer via Bluetooth. The inset shows an image of the flexible wireless electronics; (B) experiments with consumption of 12 oz of beer measured from two different human subjects, before (plot “a”) and after drinking alcohol beverage (plot “b”); (C) control experiments without drinking, first measurement (plot “a”), and second measurement (plot “b”). BAC was recorded by breathalyzer. Potential step to -0.2 V (vs Ag/AgCl).

No drinking) indicates that the sensor response is caused by the alcohol consumption. The data also indicates that the iontophoresis process does not affect amperometric current signal despite the loss of pilocarpine (and its electrolyte from the hydrogel) during the iontophoresis. The last control experiments, performed without iontophoresis (Figure 3C, No IP), show no current signal increment due to the absence of induced sweat, although BAC was spiked up to 0.018% following the alcohol ingestion. Overall, the results of the experiments described in Figure 3B and C suggests that the current output of the new biosensor system corresponds to alcohol in the iontophoresis-induced sweat and not to any other sources including interference from oxygen reduction toward PB.

Additional on-body experiments were performed to evaluate correlation between the BAC breathalyzer output and the current response of the new alcohol tattoo sensor following a serial consumption of wine. As illustrated in Figure 3D, the first current response recorded corresponded to a BAC 0.000% (before drinking); this was followed by two sets of drinking/measurement cycles using the same electrode. Following the first drink ingestion, a distinct current response was observed and the BAC turned out to be 0.025%; the BAC increased further to 0.062% after the second drinking, and also led to a larger amperometric current signal. The resulting plot (BAC v current response) is shown in Figure 3D. The current response increases linearly with the BAC level (slope, $102 \mu\text{A}/\text{BAC} \%$; correlation coefficient, $R^2 = 0.999$), indicating good correlation between the sweat ethanol level and the BAC in the same subject. The results of Figure 3B and D indicate that future efforts will focus on personalizing the sensor to different subjects. While the concept has been demonstrated in connection to the consumption of wine and beer, it can be applied to the consumption of other alcoholic beverages offering a broad noninvasive route to estimate BAC.

Characterization of an Integrated Wireless Tattoo-Based Alcohol Sensor. The practical use of the new wearable alcohol biosensor requires integration with a body-compliant wireless circuit board. As illustrated in Figure 4A, a flexible and

compact printed electronic circuitry has been developed for controlling the iontophoretic and detection processes along with a real-time wireless data transmission via a BLE radio. Details of this board are given in the Experimental Section. The performance of the integrated wearable device was evaluated following the same protocol as previous on-body experiments. A BLE-enabled printed circuit board was employed for iontophoresis by applying 0.6 mA current and amperometric measurements at -0.2 V for 150 s. The current response was sampled with a frequency of 1 Hz, and transmitted in real time via Bluetooth 4.0 to laptop/mobile devices and plotted on screen with graphical interface developed using Python. Figure 4B, C illustrates the application of the integrated flexible tattoo iontophoresis/sensor-electronic platform for on-body testing of alcohol consumption using two human subjects. Figure 4B displays the wirelessly transmitted current response and demonstrates a clear difference in the signals before (a) and after (b) consumption of the alcohol beverage. As illustrated in Figure 4C, no current response is observed in analogous on-body control experiments without drinking. This performance corresponds to that observed in the on-body data of Figure 3B, C involving the lab-scale potentiostatic analyzer. Overall, Figure 4B, C demonstrates the practicality of the skin-worn integrated flexible alcohol sensor-electronic wireless system toward noninvasive BAC monitoring.

CONCLUSIONS

We have demonstrated the first example of a completely wearable tattoo-based alcohol biosensor system, combining three important functions in a single wearable tattoo platform: sweat-inducing direct iontophoresis with amperometric enzyme biosensing, toward noninvasive alcohol monitoring from human sweat, along with a thin flexible printed electronic circuitry for controlling the entire operation and a wireless real-time data collection. The tattoo-based biosensor system provides reliable monitoring of alcohol consumption in real-world settings, as confirmed using a variety of control experiments. Such development of a low-cost single-use epidermal alcohol biosensor brings a highly useful tool for

monitoring alcohol on our highways, in crime scenes, or in the workplace. The new sensor system could thus be fitted in vehicles to measure the drivers' blood alcohol concentration, and integrated with the vehicles' alcohol ignition interlocks to prevent driving while intoxicated. Such single-use printed tattoos could be used by bartenders or friends for identifying patrons that become intoxicated and preventing drunk driving. The future systems would require additional efforts for calibrating the devices and ensuring data security and privacy safeguards. The calibration will offer real-time BAC value, and will be obtained by "personalizing" the device (to account for variability in the skin permeability and sweat composition among individuals). The attractive design and performance of the new wearable iontophoresis-sensing platform also offers considerable promise for noninvasive monitoring of additional target analytes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acssensors.6b00356](https://doi.org/10.1021/acssensors.6b00356).

Integration of the wearable alcohol tattoo sensor with wireless electronics and its operation in real time via Bluetooth communication (AVI)

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Notes

The authors declare no competing financial interest.

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