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Editors' Highlight

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Electrochemical Tattoo Biosensors for Real-Time Noninvasive Lactate Monitoring in Human Perspiration

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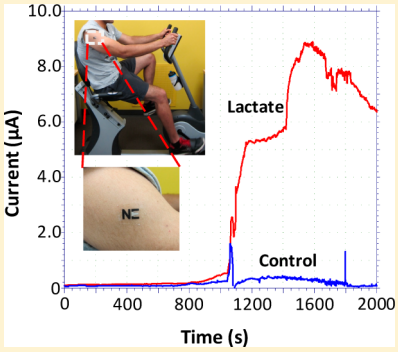
Supporting Information

ABSTRACT: The present work describes the first example of real-time noninvasive lactate sensing in human perspiration during exercise events using a flexible printed temporary-transfer tattoo electrochemical biosensor that conforms to the wearer's skin. The new skin-worn enzymatic biosensor exhibits chemical selectivity toward lactate with linearity up to 20 mM and demonstrates resiliency against continuous mechanical deformation expected from epidermal wear. The device was applied successfully to human subjects for real-time continuous monitoring of sweat lactate dynamics during prolonged cycling exercise. The resulting temporal lactate profiles reflect changes in the production of sweat lactate upon varying the exercise intensity. Such skin-worn metabolite biosensors could lead to useful insights into physical performance and overall physiological status, hence offering considerable promise for diverse sport, military, and biomedical applications.



Wearable sensors have received considerable attention since they enable continuous physiological monitoring toward maintaining an optimal health status and assessing physical performance.[1] Recent research activity in this rapidly growing field has aimed at addressing the demands of epidermal sensing where durability, lightweight, and intimate skin conformance are core requirements.[2] These endeavors have resulted in a plethora of physical sensor devices for assessing vital signs such as heart rate, respiration rate, skin temperature, bodily motion, brain activity, and blood pressure.[3] However, further progress in this arena has been hindered by the lack of wearable and conformal chemical sensors and biosensors, able to monitor the chemical constituents residing on the epidermis of the wearer's body. Such wearable chemical (bio)sensors could lead to additional important insights into the overall health status than physical variables alone can provide.

The present work demonstrates a noninvasive enzymatic temporary-transfer tattoo biosensor for the continuous monitoring of lactate in human perspiration. Lactate is one of the most important biomarkers of tissue oxygenation, and thus it is of paramount importance for assessing physical performance for sports, military, and health care applications.[4] During intense physical activity, the usual aerobic metabolism is incapable of satisfying the energy demands of the human body. This is especially true in endurance-based activities such as the triathlon, cycling, or boxing. In such instances, the anaerobic process is invoked wherein the stored glycogen is consumed to produce energy and lactate by muscle cells. This process is known as "glycolysis" or "lactate acidosis" and involves increased lactate levels in the blood.[5]

Lactate has thus been widely used by coaches, exercise physiologists, and sports physicians to monitor an athlete's performance, particularly in connection to intensive and endurance-based activities.[6] Several lactate sensor strips, which rely on finger-stick blood draws, are commercially available. However, an inherent drawback of these sensors for sport and military applications is their intrusiveness and inconvenience, especially during physical activity/exercise. To obtain a temporal lactate profile, the subject's blood is usually collected repetitively at brief time intervals while the athlete engages in rigorous training; this approach invariably hinders performance. This study seeks to tender useful information regarding an athlete's metabolic response to controlled physical activity by offering useful insights into the temporal dynamics of lactate concentration in the perspiration in a completely noninvasive manner.

Sweat lactate is a function of eccrine gland energy metabolism; an increase in the exercise intensity leads to increased production of sweat lactate.[7] Perspiration may thus be conveniently utilized for the analysis of physical performance in individuals without the need for an invasive blood sampling approach.[8] Sweat lactate can also serve as a sensitive marker of tissue viability and may provide warning for pressure ischemia, reflecting the insufficient oxidative metabolism and a compromise of tissue viability.[9]

Researchers have indeed made efforts to develop noninvasive systems for measuring lactate in perspiration.[10] However, these systems mandate the use of patches for sweat collection followed by laboratory analysis of the samples for lactate levels. Such processing is cumbersome and does not provide instantaneous feedback regarding dynamic fluctuations in lactate concentration. In the personalized healthcare field, especially in the sports/

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athletics/fitness domains, one requires a rapid and user-centric approach to analyze samples for efficient assessment. Hence, body-worn and easy-to-use sensing devices are highly desired for the continuous assessment of lactate. Khodagholy et al. recently demonstrated an electrochemical transistor-based patch that could possibly be leveraged for sweat lactate sensing directly on the human epidermis.[11] However, no real-time, on-body perspiration lactate sensing study was conducted by the researchers.

In the present work, we demonstrate a noninvasive enzymatic temporary-transfer tattoo electrochemical biosensor for the continuous assessment of lactate levels in human perspiration. In particular, the new device aims at yielding useful insight into the temporal dynamics of sweat lactate production during controlled physical activity. The development of epidermal biosensors for lactate monitoring builds on our promising recent introduction of temporary transfer tattoo-based electrochemical sensors.[12] These flexible tattoo sensors, fabricated via conventional screen printing methods, conform to the contours of the body and display resiliency toward extreme mechanical stresses expected from physical activity due to the presence of dispersed carbon fibers within the screen printed inks. The new epidermal lactate biosensor, displayed in Figure 1, consists of a mediated lactate

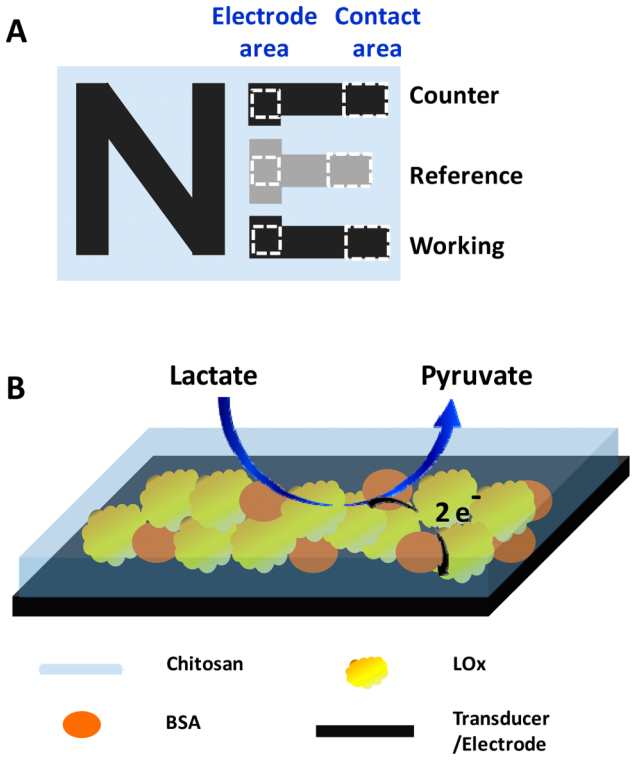

Figure 1. (A) Schematic illustration of a three-electrode "NE" tattoo biosensor for electrochemical epidermal monitoring of lactate. (B) Constituents of the reagent layer of the working electrode which is coated by biocompatible polymer (chitosan). See the text for further details.

oxidase (LOx) working electrode, prepared by functionalizing the surface of the printed tattoo electrode with tetrathiafulvalene (TTF) and multiwalled carbon nanotubes (CNT), followed by tethering the LOx enzyme, and a biocompatible chitosan overlayer. The latter prevents the efflux of the biochemical backbone from the reagent layer onto the underlying epidermis. The resulting tattoo lactate biosensor was evaluated extensively for its capability to withstand repeated iterations of mechanical deformations relevant to the wearer's daily activity. Additionally, its analytical figures of merit have been characterized. Finally, in order to validate the concept, the lactate biosensor was applied to the skin of human subjects, who were asked to endure prolonged physical exercise (cycling); the corresponding sweat lactate temporal profiles were recorded via amperometric methods. Simultaneous assessment using control epidermal tattoo sensors (lacking the LOx enzyme) confirmed the high specificity toward sweat lactate. The temporal lactate profiles demonstrate that the new wearable lactate biosensor platform performs desirably under fitness routines, thereby substantiating its utility for the noninvasive assessment of lactate levels and degree of physical exertion. Such design, characterization, and in vivo evaluation of the new printed temporary transfer tattoo epidermal lactate biosensor are described in the following sections.

EXPERIMENTAL SECTION

Reagents and Instrumentation. Glutaraldehyde solution (8%), tetrathiafulvalene (TTF), chitosan, acetic acid, bovine serum albumin (BSA), L-lactic acid, sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), D(+)-glucose, L(+)-ascorbic acid, uric acid, and creatinine were obtained from Sigma-Aldrich (St. Louis, MO). L-Lactate oxidase (LOx) (activity, 101 U/mg) was procured from Toyobo Corp. (Osaka, Japan). Carboxy-functionalized multiwalled carbon nanotubes (CNT) were purchased from cheaptubes.com (Brattleboro, VT). All reagents were used without further purification. Carbon fibers (8 μm diameter, 6.4 mm length, 93% purity) were purchased from Alfa Aesar (Ward Hill, MA), and further processing was performed to reduce their length to approximately 2 mm; the carbon fibers were subsequently rinsed with acetone. Electrochemical characterization was performed at room temperature using a CH Instruments electrochemical analyzer (model 1232A, Austin, TX). The applied potentials in all measurements were versus the screen-printed pseudo Ag/AgCl reference electrode.

Temporary Transfer Tattoo Fabrication, Electrode Modification and Transfer Process. An "NE" logo, acronym for "NanoEngineering", was utilized for the design of the sensor (Figure 1A). The fabrication process of the tattoo is similar to our earlier work with slight modifications.[12] Briefly, chopped carbon fibers were dispersed within both conductive carbon (E3449) and silver/silver chloride (E2414) inks (Ercon Inc., Wareham, MA) to 1.5 and 1.2 wt % levels, respectively, to increase the tensile strength of the electrodes. Sensor patterns were designed in AutoCAD (Autodesk, San Rafael, CA) and outsourced for fabrication on stainless steel through-hole 12 in. × 12 in. framed stencils (Metal Etch Services, San Marcos, CA). Papilio temporary transfer tattoo base paper was purchased from HPS LLC (Rhome, TX). A sequence of the aforementioned carbon, silver, and insulator (Dupont 5036, Wilmington, DE) inks were patterned on the substrate employing an MPM-SPM semi-automatic screen printer (Speedline Technologies, Franklin, MA). As shown in Figure 1A, the "E" portion of the tattoo sensor design consists of a pseudoreference (silver/silver chloride ink), counter and working electrodes (carbon ink). A transparent insulator was screen printed on the surface of the electrode pattern to confine the electrode and contact areas. Following every screen printing step, the printed pattern on the temporary transfer tattoo paper was cured at 90 °C for 15 min in a convection oven.

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Following the printing of the tattoo electrode transducers, the working electrode was functionalized with the reagent layer. CNT were first suspended in ethanol (5 mg mL^{-1}) and sonicated for several hours until a uniform suspension was achieved. The suspension was subsequently mixed with a 0.1 M TTF ethanol/acetone (9:1 (v/v)) solution, using a volume ratio of 2:1 and sonicated for 1 h. A volume of 3 μL of the CNT/TTF suspension was subsequently cast onto the open area of the working electrode and was allowed to air-dry. Following this, 3 μL of LOx solution (40 mg mL^{-1} with 10 mg mL^{-1} of the BSA stabilizer) was cast on the electrode and dried under ambient conditions; this surface was later covered with 2 μL of a 1 wt % chitosan solution prepared in 1 wt % acetic acid. The electrodes were finally cross-linked with glutaraldehyde vapor in a sealed chamber and maintained at 4 °C overnight.

The temporary tattoo transfer process has been previously reported.[12a] The one used in this study was identical with a minor modification made, a gap (12 mm × 3.5 mm) was included around the electrode area (between the tattoo and the skin) to facilitate the flux of fresh perspiration over the sensor for proper replenishment essential for continuous epidermal evaluation. With respect to in vitro amperometric measurements, the tattoos were applied to the substrate such that their functionalized side faced upward. In contrast, during epidermal evaluation, the functionalized side faced downward (making contact with the epidermis).

In Vitro Evaluation. The electrochemical performance of the tattoo lactate sensor was evaluated in vitro by transferring it onto a rigid plastic substrate or onto a flexible GORE-TEX textile for mechanical integrity studies. These studies were performed using a 0.1 M phosphate buffer (pH 7.0) solution. The operating potential for the tattoo lactate sensor was selected by using linear sweep voltammetry with a scan rate of 1 mV/s from −0.2 to 0.2 V using 8 mM lactate. The amperometric response was recorded after a 1 min incubation in the sample solution, using a potential step to +0.05 V (vs Ag/AgCl) for 60 s. Mechanical strain studies were performed with the transferred tattoo sensor on a GORE-TEX textile to emulate the viscoelastic properties of the skin. The biosensor was thus stretched ∼10% and bent at 90° for 5 s followed by subsequent relaxation for another 5 s. The bending/stretching and subsequent relaxation of the sensors was iterated 10 times after which the response to 8 mM lactate was measured. The sensor specificity was examined in the presence of physiological levels of the relevant constituents of human sweat, namely, 84 μM creatinine, 10 μM ascorbic acid, 0.17 mM glucose, and 59 μM uric acid.[13]

Epidermal Lactate Sensing. The epidermal biosensor evaluation was performed in strict compliance with the protocol that was approved by the institutional review board (IRB) at the University of California, San Diego. The study was deemed by the IRB as posing "no greater than minimal risk" to the prescreened subjects who were recruited for the investigation. A total of 10 healthy volunteers (7 males and 3 females between the ages of 20 and 40, recruited in response to follow-up from flyers) with no prior medical history of heart conditions, diabetes, or chronic skeletomuscular pain were recruited for participation in the study, and informed, signed consent was obtained from each individual following a rigorous prescreening procedure. A tattoo lactate sensor was applied on the volunteer's deltoid in order to assess the real-time lactate concentration profile. Rectangular sections of polyethylene terephthalate (PET) (3 mm × 18 mm), screen-printed with carbon ink, were attached to the tattoo contact pads (using conductive silver epoxy); this was used as a low-noise interface to the electrochemical analyzer, in connection to fine stainless steel wires (Figure S1 in the Supporting Information). The lactate response was recorded using amperometry at a low potential of +0.05 V (vs Ag/AgCl), which substantially reduces the possibility of electric shock due to the diminished electromotive force. Moreover, the potentiostat was configured with a current limit of 100 μA (the threshold for human perception and maximum safe level recommended by the U.S. National Electric Code) in order to diminish the chance of electric shock (***Safety note**: It is highly advised that the amperometric measurement apparatus be **voltage- and current-limited** in order to reduce the likelihood of unintentional electric shock*). In order to validate the selectivity of the biosensor to oxidize lactate, two temporary transfer tattoos were applied on the subjects' deltoid, one containing the LOx enzyme and the other absent of this enzyme.

Subjects were asked to mount a stationary cycle and begin cycling at a steady, comfortable cadence. Subjects were instructed to maintain their cadence while an increasing resistance was applied at 3 min intervals. The absolute resistance level was selected according to subject's fitness level while the same intensity profile was used throughout the human studies. This ensured that the anaerobic metabolism was invoked at similar time scales, hence augmenting the excretion of lactate in the perspiration in a controlled fashion. Following the intense fitness bout, the volunteers were asked to gradually reduce their cadence during a 3 min "cool-down" period whereby the resistance was reduced from maximal levels.

RESULTS AND DISCUSSION

Rationale for CNT/TTF/LOx/Chit Surface Functionalization. The lactate concentration of the human sweat depends on a person's metabolism and physical performance and usually varies up to 25 mM.[14] Thus, a wide linear detection range coupled with a fast response time is essential for continuous epidermal monitoring of lactate. The tattoo sensors have been designed to meet these requirements, while eliminating the risk of skin exposure to the constituents of the reagent layer (and potential toxic effects). Typical lactate biosensors utilize lactate dehydrogenase (LDH) or lactate oxidase (LOx). However, LDH requires the NAD^+ cofactor, which represents a noteworthy challenge for continuous noninvasive monitoring applications. LOx-based amperometric detection of lactate commonly requires a relatively high potential (>+0.65 V) in order to monitor the liberated peroxide product and is subject to potential electroactive interferences. Mediators, such as TTF, have been used to address such interferences by facilitating the low-potential electrocatalytic conversion of lactate by LOx.[15] TTF has been widely used as a mediator for biosensor applications and was shown not to cause skin irritation.[16] To improve the efficiency of the mediated tattoo lactate biosensor, CNT were dispersed together with TTF to serve as an effective electron transducer on the working electrode. The CNT/TTF complex was employed previously as an efficient electron shuttle.[17] Furthermore, given the goal of continuous epidermal usage of the tattoo sensor, the CNT/TTF/LOx reagent layer was coated with a biocompatible chitosan overlayer that functioned as a physical barrier and limited the efflux of the catalytic backbone from the tattoo onto the underlying epidermis.

In Vitro Evaluations. To determine the operating potential of the tattoo sensor, linear sweep voltammetry was employed first in the presence and absence of lactate in buffer. The sensor displayed an onset potential of ∼−0.15 V with a peak around

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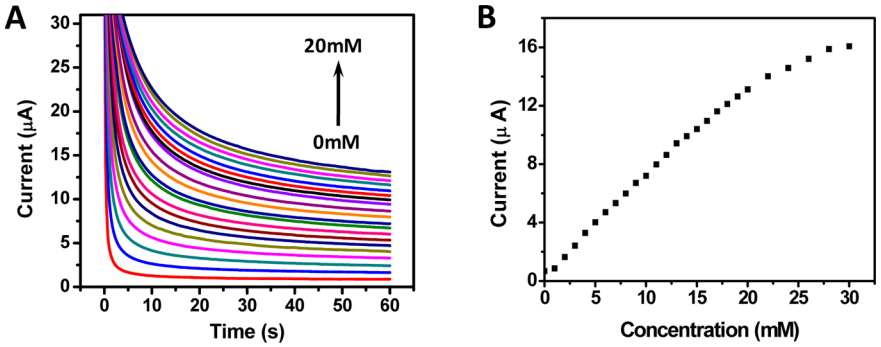

Figure 2. (A) Amperometric response for increasing concentrations of lactate (1 mM increments) over the 0−20 mM range; $E_{applied}$ = +0.05 V (vs Ag/AgCl). (B) Calibration plot up to 30 mM lactate, based on sampling the current at 60 s.

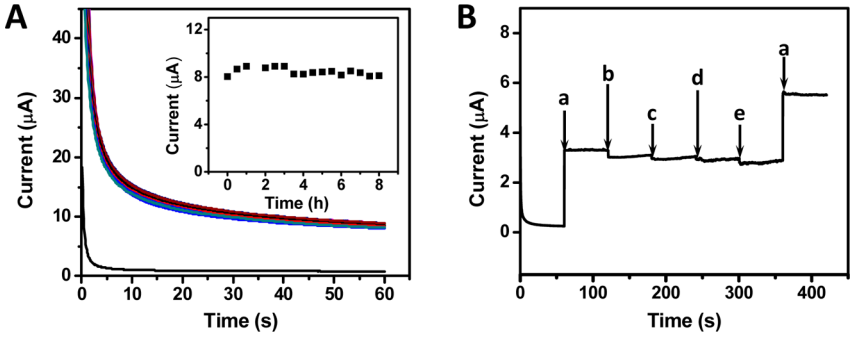

Figure 3. (A) Stability of the response of the tattoo biosensor to 8 mM lactate at 30 min intervals over a 8 h period. The inset is the corresponding current−time plot of the chronoamperometric response. The tattoo sensor kept at ambient conditions between such successive runs. (B) Selectivity study: Response to (a) 4 mM lactate, (b) 84 μM creatinine, (c) 10 μM ascorbic acid, (d) 0.17 mM glucose, and (e) 59 μM uric acid at +0.05 V (vs Ag/AgCl).

+0.05 V (vs Ag/AgCl) for the oxidation of lactate (not shown), indicating that the CNT/TTF/LOx/Chit reagent layer offers low potential lactate oxidation. Such low potential oxidation reflects the efficient electron donor−acceptor interaction of CNT/TTF,[18] which supports the shuttling of electrons between the redox center of the enzyme and the electrode surface. A potential step to +0.05 V (vs Ag/AgCl) was thus selected for all subsequent chronoamperometric measurements. The dynamic concentration range in response to increasing levels of lactate was subsequently investigated. Figure 2A displays chronoamperograms obtained with the LOx-functionalized tattoo sensor for increasing lactate concentrations at 1 mM increments in 0.1 M phosphate buffer (pH 7.0). The tattoo sensor exhibits a well-defined lactate concentration dependence, with a highly linear response throughout the 1 mM to 20 mM range, beyond which a very slight curvature is observed (Figure 2B). Notice that the lactate concentration in human sweat usually varies to maximal levels of 25 mM.[14] The linear dynamic range is characterized with high sensitivity (slope, 644.2 nA/mM or 10.31 μA/mM cm^2; correlation coefficient, 0.996). Such high sensitivity is indicated also from the well-defined response to 1 mM lactate (Figure 2A) and the corresponding low noise level.

Practical continuous monitoring applications of the new epidermal lactate biosensor paradigm require high operational stability during prolonged periods of operation. The stability of the tattoo biosensor was examined from the response to 8 mM lactate over an 8 h period, wherein the response of the tattoo sensor was recorded every 30 min. As illustrated from the repetitive chronoamperograms provided in Figure 3A, the lactate biosensor yields highly reproducible results (RSD = 3.60%), thus underscoring its applicability for long-term epidermal use. The highly stable response reflects the integrity of the tattoo biosensor and the constituents of its reagent layers, benefit from the water-insoluble TTF mediator, the BSA enzyme stabilizer, a protective chitosan overcoating, and the glutaraldehyde cross-linker. Apparently, changes in the sweat pH and lactate levels have no apparent effect upon the enzyme activity, reflecting the "protective action" provided by the constituents of the reagent-layer. The shelf life of the biosensor was also examined for the ones stored at 4 °C for a period of 5 months. The responses of the biosensor remained stable during this prolonged storage period, with less than a 10% decay of the sensitivity.

The specificity of the biosensor was further examined by considering that perspiration consists of a plethora of metabolites and electrolytes. Among these constituents, ascorbic acid, uric acid, glucose, and creatinine can affect the response of the sensor and lead to inaccurate readings. The tattoo biosensors were thus evaluated in the presence of these interfering agents at physiological levels found in the perspiration. As shown in Figure 3B, the device responds favorably and rapidly to 4 mM lactate, while the contributions imparted by the selected interfering agents were negligible (less than 5% compared to the response associated with lactate). The high selectivity is also illustrated below from control experiments employing an epidermal sensor lacking the LOx enzyme. Such high selectivity reflects the low operating potential and the composition of the reagent layer.

The human epidermis regularly experiences deformations due to bodily movements. Such epidermal deformations are a major cause of concern for wearable devices wherein the devices undergo disfigurations similar to the skin. This is especially true

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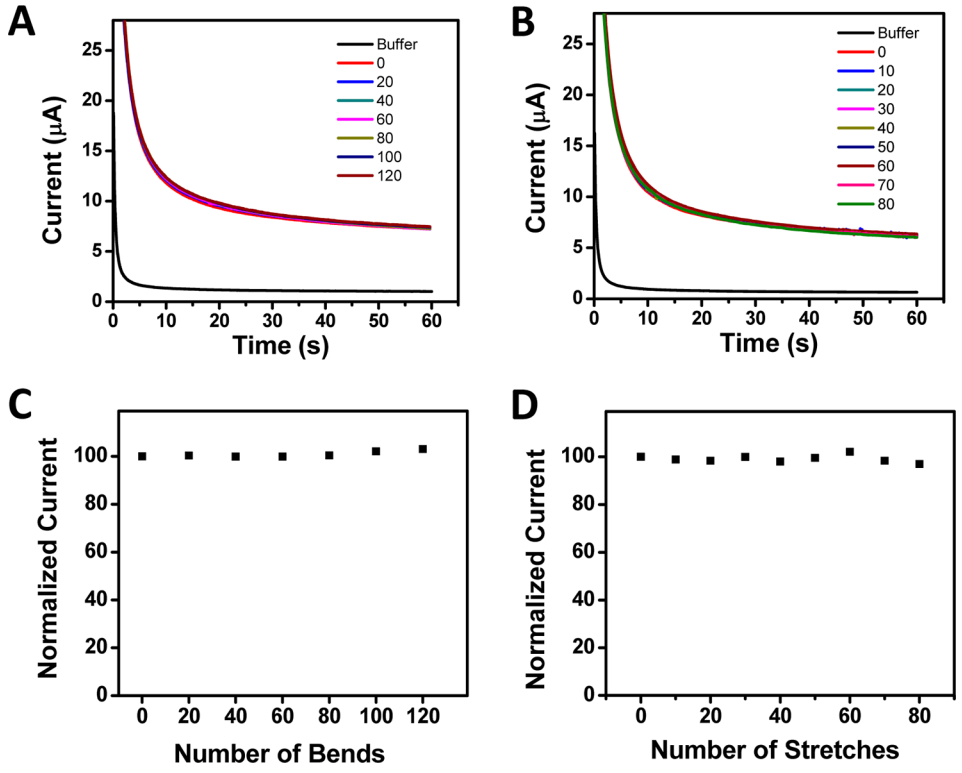

Figure 4. Electrochemical response of tattoo lactate sensor transferred onto a flexible GORE-TEX textile undergoing repeated bending (A) and stretching (B) to 8 mM lactate at +0.05 V (vs Ag/AgCl) and their normalized current (C) and (D), respectively.

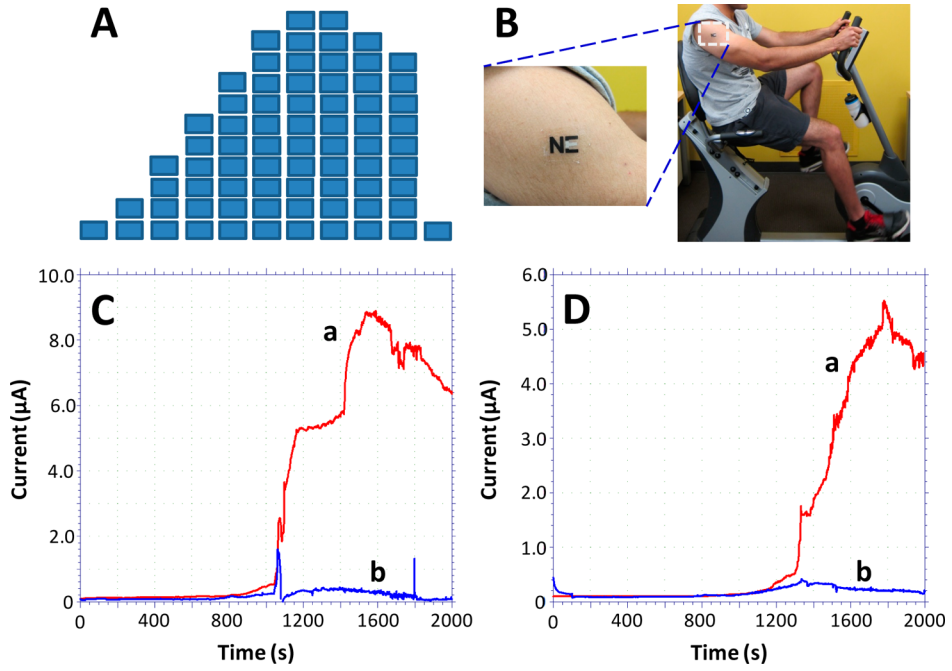

Figure 5. Monitoring of sweat lactate during 33 min of cycling exercise while changing the work intensity. (A) Exercise resistance profile on a stationary cycle. Subjects were asked to maintain a constant cycling rate while the resistance was increased every 3 min for a total evaluation of 30 min. A 3-min cool down period followed the exercise. (B) An "NE" lactate biosensor applied to a male volunteer's deltoid; (C and D) Response of the LOx- (a) and enzyme-free (b) tattoo biosensors during the exercise regimen (shown in part A) using two representative subjects. Constant potential, +0.05 V (vs Ag/AgCl); measurement intervals, 1 s.

for epidermal sensors since these devices contact the epidermis directly. As the human body engages in locomotion, the epidermal layer can undergo bending, stretching, and twisting stresses. Accordingly, the robustness of the temporary transfer tattoo sensor was evaluated by applying it to flexible GORE-TEX textile. The temporary transfer tattoo sensor was flexed to a 90° angle for 120 iterations; the sensor response was recorded every 20 bending iterations. This was followed by stretching the same

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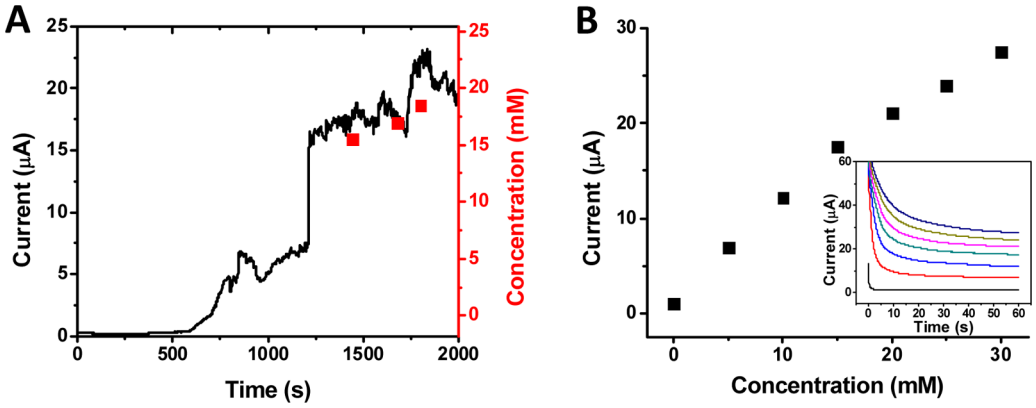

Figure 6. (A) Real-time response of the tattoo biosensor during a cycling exercise (left *y*-axis) and corresponding lactate concentrations (right *y*-axis); red dots represent the lactate concentrations in the sweat collected at these times. (B) In vitro calibration curve of tattoo biosensor at 37 °C; inset, amperometric response to different lactate concentrations up to 30 mM with 5 mM increments.

sensor by 10% for a total of 80 iterations; data was recorded every 10 stretching iterations. Each bending/stretching cycle consisted of bending or stretching for 5 s followed by a 5 s relaxation period. As indicated from Figure 4, the response of the temporary transfer tattoo sensor in both the bending (part A) and stretching (part B) studies remained highly uniform, and the corresponding normalized data (parts C and D) are highly stable with an RSD of 1.24% and 1.50%, respectively. The minimal deviation of the sensor response even after subjecting it to a large number of stress cycles can be attributed to the dispersed carbon fibers within the carbon and Ag/AgCl inks, which provide an interleaved conductive backbone. The transparent insulator, covering most of the sensor surface, further enhances the structural integrity (by facilitating adhesion among the printed pattern). As will be illustrated in the following epidermal evaluations, the resulting temporary transfer tattoo lactate biosensor performs desirably in the face of various mechanical stressors characteristic to epidermal operation

Epidermal Evaluation. Prior to epidermal lactate-sensing evaluation, the temporary transfer tattoo biosensors were examined for their ability to adhere to the epidermal surface under various forms of mechanical strain. Visual evaluation of a temporary transfer tattoo biosensor on the human neck region was performed in connection to different strain deformations. The device underwent repeated stretching, bending, and twisting stressors for a total of 100 iterations each. Figure S2 in the Supporting Information displays images of the sensor during these studies. The left column provides a top-view of the device during the various deformations. The right column displays photos of the devices at the conclusion of each study. The images clearly demonstrate that the tattoo biosensors are quite resilient to these flexions.

Proceeding with the operational characterization of the devices under practical scenarios, real-time epidermal lactate levels in 10 consenting subjects (recruited in strict compliance with IRB protocols delineated in the Experimental Section) were monitored utilizing the temporary transfer tattoo biosensors. Dynamic changes in sweat lactate levels were thus measured continuously during a 30 min bout of intense cycling while the exercise resistance/intensity was adjusted in pre-established intervals. Specifically, in order to ensure that the anaerobic metabolism was invoked, subjects were asked to mount a stationary cycle and maintain a steady cycling cadence while the resistance/intensity profile, illustrated in Figure 5A, was instituted. The temporary transfer-tattoo biosensor was applied to the volunteer's right deltoid (Figure 5B), and a custom-designed thin-film flexible connector was utilized to interface the three-electrode biosensor with a hand-held electrochemical analyzer. Medical grade adhesive was utilized to secure the electrical connection to the surface of the sensor connectors (Figure S1 in the Supporting Information). In order to validate the ability of the biosensor to monitor lactate selectively, two temporary transfer tattoos were applied simultaneously on the subjects' deltoid in close vicinity, one containing the LOx enzyme and the other absent of this enzyme (serving as the control).

Figure 5C,D displays the continuous raw amperometric data (screen captures from the recording instrumentation without any postprocessing) of two representative subjects, among the larger group of volunteers. In each figure, the output of the LOx-functionalized (a, red) and control "enzyme-free" (b, blue) tattoo biosensors are overlaid. As indicated from these plots, unlike the control sensors, the LOx-functionalized biosensors displayed facile biocatalytic ability toward lactate oxidation in the perspiration. Moreover, the background current level and the corresponding noise level are minimal. The extremely low noise level, observed directly on the body, reflects the quality of the amperometric signal transduction and corresponding electrical contacts. During the initial period of the cycling exercise, no apparent faradaic current is observed at both electrodes, reflecting the lack of perspiration present on the epidermis. As the exercise ensued, the skin became moist (albeit sweat formation was not yet observed), imparting electrical conductivity at the sensing surface, thereby contributing to the slow current rise observed at 850 and 1150 s, for subjects C and D, respectively. Additional sharper increases in the background current are observed only at the moment that the subject began to perspire (around 1000 and 1250 s for subjects C and D, respectively). At this point, owing to an electrolytic fluid (sweat) covering the biosensor surface, the lactate level could be assessed. Furthermore and more interestingly, for subject C, a lactate threshold, corresponding to the buildup of lactate in the perspiration at the transition from aerobic to anaerobic respiration, is clearly observed at ~1450 s.[19] Subject D, on the other hand, a notably less "fit" individual, demonstrated such a transition earlier in their fitness routine, shortly after the onset of sweating at ~1350 s. It should be noted that for both cases, the observed temporal profiles, corresponding to instantaneous lactate readings in the perspiration, reflect the resistance/intensity profile (of Figure 5A) and hence substantiate that the sweat lactate levels change dynamically in response to varying

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levels of physical exertion. Similar current−time profiles were obtained among the other subjects, all of whom demonstrated the onset of the lactate threshold during the controlled fitness routine, albeit at different time scales, reflective of their respective aerobic capacity.[20] The final stage (over the last 3 min) of the controlled exercise bout consisted of a cool-down. During this period, the subjects cycled with less intensity and were encouraged to reduce their cadence to a comfortable rate.

The epidermal amperometric data, shown in Figure 5C,D, reveal a similar pattern where a slight decrease in the signal is observed during the cool-down period, hence substantiating that the observed epidermal lactate profiles closely track the exercise intensity with near instantaneous response. In contrast, the "control" tattoo sensors (without LOx) worn by the two subjects display negligible current fluctuations throughout the entire exercise regimen (b), reflecting the absence of biocatalytic activity. The lack of response at the "enzyme-free" sensors is also indicative of the remarkable selectivity of the tattoo sensing device and its intrinsic ability to address potential interference from electroactive constituents present in the perspiration. The results, therefore, clearly support that the profiles observed at the LOx-functionalized temporary-transfer tattoo biosensors are solely due to dynamic changes in sweat lactate levels associated with the exercise intensity. Such attractive performance and high selectivity make the new epidermal biosensor extremely attractive for assessing the wearer's physical exertion and fitness. In addition, no signs of skin irritation or inflammatory response were observed among the subjects following the exercise.

To further validate the lactate excretion profile, sweat samples were collected from a volunteer during cycling. As illustrated in Figure 6A, a similar lactate profile was obtained for an additional subject with a lactate rise onset time of less than 10 min due to the high exercise intensity. A sharp increase in the response was observed at 20 min, possibly reflecting the greater effort to maintain the speed in the wake of increasing exercise resistance/intensity. In order to estimate the real-time lactate concentration, the biosensor was calibrated at 37 °C to emulate it's effect of the physiological temperature on the enzyme's activity (Figure 6B). As expected, the sensitivity of the biosensor was approximately 1.4 times greater (s_x = 0.916 μA/mM or 14.66 μA/mM cm^2) than that measured at room temperature. Accordingly, the resulting calibration curve (of Figure 6B) can be leveraged to correlate the measured current during epidermal evaluation to the absolute lactate concentration (as shown in Figure 6A). To corroborate the accuracy of the biosensor, sweat samples were collected from the volunteer's deltoid at approximately 24, 28, and 30 min following the initialization of the exercise routine, and their lactate level was examined at 37 °C with a new tattoo biosensor. The corresponding lactate concentrations, shown as red marks in Figure 6A, indicate a strong correlation between the in vivo response and the in vitro data (~10−15% deviation). Sweat samples from another volunteer were also examined in the same fashion and a ~6% deviation was observed, further validating the sensor's epidermal response. Such small variation between the in vivo and in vitro data may be attributed to the perspiration collection methodology where sweat was collected over a 1 min duration, leading to an integrated lactate in vitro response rather than an instantaneous one, as measured in vivo.

CONCLUSIONS

We have demonstrated the first example of an epidermal electrochemical biosensor which provides real-time analysis of sweat lactate during exercise. Such direct epidermal monitoring of lactate has been realized through the use of flexible printed temporary-transferred tattoos functionalized with lactate oxidase. The resulting epidermal data has been shown to monitor sweat lactate dynamics closely tracking exercise intensity. Compared with traditional blood draws for lactate, the epidermal biosensor is noninvasive, is simple-to-operate, and causes no hindrance to the wearer. Furthermore, the tattoo biosensors endure repetitive mechanical deformations experienced by the epidermis during exercise. Future efforts are aimed at further miniaturization and integration of the electronic interface, data processing, and wireless transmission of the results. While the on-body results gathered in this study are preliminary in scope, future efforts will be directed at more detailed physiological studies. Such studies will account for differences in temperature and relative humidity in conjunction with a larger cohort of subjects in order to assess the utility of the epidermal sensing concept for assessing physical performance among the general population. Moreover, future studies will seek to concurrently correlate lactate levels measured in the perspiration with those measured in the blood during a controlled fitness routine. In order to realize ubiquitous sensing, future efforts will also migrate the electronics required for control and readout of the sensor onto the same temporary transfer tattoo substrate. The new amperometric epidermal biosensing concept can readily be expanded toward skin-worn monitoring of other clinically relevant sweat metabolites and could thus find important applications for athlete or soldier performance assessment as well as in the generalized healthcare domain.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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