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A Wearable Patch for Prolonged Sweat Lactate Harvesting and Sensing

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***Abstract*— Operating at low sweat rates, such as those experienced by humans at rest, is still an unmet need for state-of-the-art wearable sweat harvesting and testing devices for lactate. Here, we report the on-skin performance of a non-invasive wearable sweat sampling patch that can harvest sweat at rest, during exercise, and post-exercise. The patch simultaneously uses osmosis and evaporation for long-term (several hours) sampling of sweat. Osmotic sweat withdrawal is achieved by skin-interfacing a hydrogel containing a concentrated solute. The gel interfaces with a paper strip that transports the fluid via wicking and evaporation. Proof of concept results show that the patch was able to sample sweat during resting and post-exercise conditions, where the lactate concentration was successfully quantified. The patch detected the increase in sweat lactate levels during medium level exercise. Blood lactate remained invariant with exercise as expected. We also developed a continuous sensing version of the patch by including enzymatic electrochemical sensors. Such a battery-free, passive, wearable sweat sampling patch can potentially provide useful information about the human metabolic activity.**

***Clinical Relevance*— Lactate is an important biomarker for determining oxidative stress levels, cardiorespiratory performance, and muscle health. Its monitoring is of prime importance, especially for those who are frequently prone to oxygen deficit conditions like athletes, military personnel (for improved training, performance, and endurance) and woman during childbirth (hypoxic distress during labor in the fetus). Critical sweat lactate levels in the body can abruptly alter fluid pH and cause several detrimental effects on human health like muscle acidosis, tissue hypoxia, sepsis, and cerebral stroke.**

I. INTRODUCTION

Lactate is a byproduct of anaerobic glycolysis, which is created due to an increased energy demand in the body. Such high energy demand is generally a consequential outcome of extensive physical exertion. Lactate is an important biomarker for determining oxidative stress levels, muscle health and tissue hypoxia [1,2]. It is predominantly present in sweat, blood [3], and to a certain extent in tear [4] and saliva [5]. The concentration of lactate in these biofluids is highly dependent on the physiological state and the site of anaerobic metabolism in the human body.

Lactate content in sweat depends on several parameters like the exercise type, duration, intensity, sweat rate [6], age, sex [7], and the sampling location due to varying sweat gland density [8]. The blood lactate stays relatively invariant under low to medium levels of exercise (60-70% of maximum heart rate (HR_{max})) and does not show a good correlation to sweat lactate levels under similar conditions [9]. A good correlation between blood and sweat lactate has been observed only during high intensity exercise (> 85% HR_{max}) [1]. Overall, the extent of lactate generation in both sweat and blood are well characterized under active sweating conditions. However, there is a need for studies to provide a comprehensive understanding of sweat and blood lactate metabolism under varying physiological conditions, such as rest, various levels of exercise, and post-exercise.

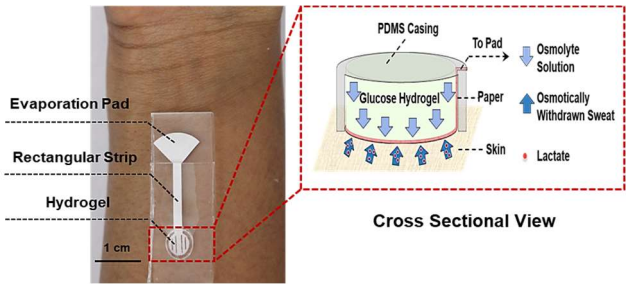

Figure 1: Image and schematic of the wearable, zero-powered, and non-invasive osmotic sweat sampling platform interfacing the skin.

The earlier work in the literature has quantified sweat and blood lactate levels using various (and mostly colorimetric and electrochemical) sensing mechanisms [4,6,10,11]. These sensing platforms function in presence of an enzyme, either lactate oxidase (LO_x) or lactate dehydrogenase (LDH). Despite delivering on-spot sweat lactate detection with high sensitivity, wide linear dynamic range, and low detection limit, the current sensing platforms face the challenges of (1) remaining inoperative under low sweating conditions; (2) being incapable of a comprehensive analysis of the human lactate metabolism and (3) requiring an external power source for continuous operation.

Herein, we report the on-skin performance of a wearable, non-invasive sweat lactate sampling wearable patch with a hydrogel skin interface (Fig. 1). The patch deploys osmosis, capillary wicking, and evaporation simultaneously for long-term (several hours) sweat lactate harvesting. It can function under low sweating conditions and without a need for an external electrical power. We initially discuss the functioning of the patch on-skin and how it harvests sweat

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lactate. This is followed by a comparative analysis of sweat lactate intake between a hypertonic hydrogel (equilibrated in 4 M glucose) and an isotonic hydrogel (equilibrated in phosphate buffer saline, PBS) embedded in the patch. Next, we track the correlation between sweat and blood lactate under varying physiological conditions. Finally, we discuss a continuous sweat lactate sensing platform based on our patch and using enzymatic electrochemical sensors.

II. EXPERIMENTAL PROCEDURE

A. Principle of Operation

Fig.1 shows the components of the patch and how the hydrogel pump functions upon interfacing with skin. The patch has three major sections: (1) a circular chamber hosting a hydrogel disk; (2) a rectangular strip of paper sandwiched between the two thin PDMS sheets; and (3) a paper pad with a large surface area (evaporation pad). The hydrogel disk is equilibrated with varying osmolytes to lower its chemical potential (of water in the gel) relative to sweat. The infused hydrogel is then loaded onto the designated circular chamber of the patch. The hydrogel disk and the circular terminus of the paper directly interface the skin. The hydrogel withdraws sweat and lactate from the skin surface due to a high osmotic pressure. The extracted sweat and lactate from skin, together with some of the osmolyte solution from the hydrogel, then, get wicked through the rectangular paper strip "channel" towards the evaporation pad for accumulation over time.

Due to continuous fluid evaporation, the pad acts as a repository of any solute and lactate dissolved in the sweat [12-14]. The evaporation of water from the pad maintains the capillary pressure in the paper strip and promotes continuous long-term fluid flow towards the pad. The patch would cease to operate (stop withdrawing fluid) if either of the following conditions are reached over time: (1) chemical potential of the water in the sweat equals the chemical potential of water in the hydrogel or (2) saturation of the pad by deposited salt / osmolyte. Hence, the useful duration of patch operation depends on both the osmotic strength of the hydrogel and the free available surface area on the pad.

B. Patch Fabrication and Hydrogel Synthesis

Sylgard-184 silicone elastomer (PDMS, Dow Corning) and its curing agent were mixed in 10:1 w/w ratio and cured for 12 hours at 70ºC to make the base for the PDMS sheet. The patch was prepared by attaching two PDMS sheets together – 38 × 15 × 2 mm^3 (bottom) and 30 × 15 × 1 mm^3 (top). A single hole matching the hydrogel diameter was punched at 6 mm from one of the edges on the bottom sheet to encase the hydrogel. A section of Whatman 542 paper was cut out using a CO_2 laser cutter (Universal Laser Systems VLS 3.5) and was sandwiched between the top and bottom PDMS sheets. The PDMS sheets were attached together using additional silicone precursor (Sylgard-184), making sure that it did not contact the paper channel. The whole patch was treated in an oven at 40ºC overnight to achieve firm adhesion between the sheets.

The hydrogels were made using acrylamide monomer, N-N' methylenebisacrylamide as the crosslinker and 2-hydroxy-4′-(2-hydroxyethoxy)-2-methyl-propiophenone as the photo-initiator. The monomer solution contained 22% acrylamide, 0.48% crosslinker and 0.15% photo-initiator (all w/w). The solution was cured inside a circular petri dish (47 mm diameter) under a 175 mW/cm^2 UV lamp (Sunray 400-SM) for 3 minutes. Disks of varying diameters were punched out and stored in either 4 M glucose or PBS for 24 hours. These solutions served as the osmolytes. After 24 hours, the infused disks were transferred to a fresh solution in a vial and stored for further usage. A single disk was taken out, blotted with a paper napkin, and was fitted inside the circular space in the patch before usage.

C. Human Testing Protocol and Statistical Analysis

Three healthy subjects, aged 20-28, were recruited at the NC State University campus and consented before the study. Preclinical human experiments were conducted during three physiological stages: (1) at rest (*i.e.* no exposure to any physical activity) for 2 hours, (2) medium intensity exercise (75-100 W) for a period of 1 hour, and (3) after 2 hours post-exercise. We used two types of patches for our human testing with two type of hydrogel equilibrated in two different osmolytes: 4 M glucose and PBS. Both the forearms were initially washed with isopropyl alcohol and DI water before snapping the patch. Blood lactate was measured after the completion of test in each physiological condition. All trials were conducted under ambient conditions (22ºC, 45% relative humidity (RH)) and strictly as per the approved IRB protocol (UNC-18-1959). We performed a two-sample t-test for statistical significance. Significance is denoted as $p < 0.05$.

D. Electrochemical Sensing Platform for Sweat Lactate

The lactate sensor was fabricated on a 50 µm thick polyimide sheet. Working electrode (WE, carbon ink with Prussian blue mediator), reference electrode (RE, Ag/AgCl), and counter electrode (CE, carbon ink) were screen-printed on the film with a 230-mesh size mask. The dimensions of all the electrodes were 1.5×2 mm^2. 15 mg/mL graphene stock solution was prepared in ethanol and sonicated in the ultrasonic bath for 60 minutes. 5 µL of an aqueous mixture (4 µL of 50 mg/mL LO_x in 1×PBS and 1 µL of 15 mg/mL graphene in 1×PBS) was drop-casted on the WE and dried at room temperature for 1 hour. 1 µL of 0.5 wt% Nafion was drop- casted on the WE and dried at room temperature for 1 hour. Chronoamperometric tests were performed with a benchtop potentiostat (600+, Gamry Inc.) at −0.1 V with respect to the RE.

III. RESULTS and DISCUSSION

A. *Testing of Hydrogels of Varying Osmotic Strengths*

We compared the *in-vivo* on-skin performance of a 4 M glucose hydrogel patch to a PBS-infused hydrogel in our preclinical human testing. The PBS hydrogel patch is isotonic to the sweat and served as a control to distinguish the osmotic contribution from 4 M glucose hydrogel. We affixed these two types of patches concurrently on both arms of three subjects at rest for 2 hours: 1 hour during medium intensity exercise, and 2 hours after exercise in a

separate set of experiments. A colorimetric assay quantified the amount of lactate collected on paper [14].

Fig.1 presents an optical image of the wearable patch on subject's forearm skin and a schematic of ow sweat lactate is withdrawn at the paper-skin interface. The glucose solution (from the 4 M glucose hydrogel) and the osmotically withdrawn sweat and lactate from skin, collectively appear in the form of a colorless liquid that wicks onto the rectangular section of the paper channel over time. The PBS hydrogel did not show any visible fluid flow in the channel. The total sampled liquid volume on the paper of 4 M glucose hydrogel patch during rest was ~ 1.6 ± 0.1 µL. During exercise, ~ 3.16 ± 1.5 µL was sampled (an additional ~ 1.5-2 µL of fluid because of the exercise). Approximately 1.43 ± 0.2 µL of fluid was sampled during post-exercise tests by this 4 M glucose hydrogel patch.

The 4 M glucose hydrogel sampled ~ 13.5 nmoles of lactate after 2 hours of testing (Fig. 2left) which corresponds to an average concentration of ~ 7 mM at rest (Fig. 2right). This lactate concentration is considered "low" as it falls towards the lower end of the normal human sweat lactate range [8,15]. The PBS hydrogel sampled ~ 11.6 nmoles of lactate after 2 hours of testing at rest. PBS is isotonic to human sweat with no osmotic withdrawing power. Hence, the traces of lactate in the paper of the PBS hydrogel patch are possibly derived from the wet extraction of the skin-held naturally generated sweat. Lower lactate sampling level by PBS hydrogel justifies the use of the 4 M glucose hydrogel patch for withdrawal of additional lactate via osmosis.

The lactate content in sweat rises with exercise as the energy demand in the body and the aerobic glycolysis rate increase. Anaerobic glycolysis compensates some of this high energy demand and increases the production of lactate (byproduct of anaerobic glycolysis), which eventually appears in sweat [2,9]. The 4 M glucose hydrogel sampled ~ 52.0 nmoles (Fig. 2left) of lactate after 1 hour of exercise, which corresponds to an average concentration of ~ 23.0 mM in the 4 M glucose hydrogel patch (Fig. 2right). Thus, the 4 M glucose hydrogel patch can detect the increment in lactate levels during exercise. The PBS hydrogel patch sampled ~ 31.0 nmoles of lactate after an hour of exercise. The PBS hydrogel patch also detects the increment in lactate levels with exercise. This higher lactate (with respect to the lactate levels at rest) in a PBS hydrogel patch is derived mainly from the inflow of active sweat. There is active

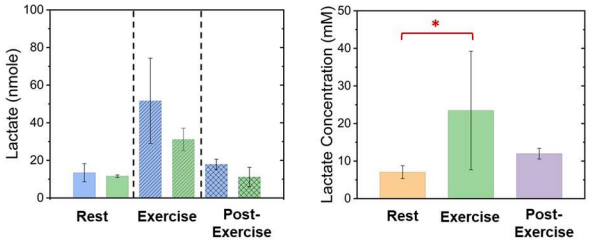

Figure 2: The average sweat lactate amount and concentration during resting, exercise, and post-exercise. (Left) A comparative analysis of the lactate sampled by 4 M glucose patch (blue) and PBS hydrogel patch (green). (Right) The change in sweat lactate concentration using a 4 M glucose hydrogel patch with exercise *p<0.05.

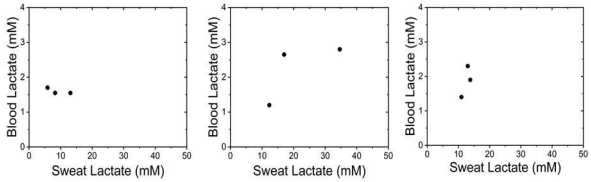

Figure 3: Variation of blood lactate concentration with sweat lactate concentration during resting (left), exercise (middle), and post-exercise (right). Each circular point denotes a different subject.

sweat inflow (~ 1.5-2 µL) in both 4 M glucose hydrogel and PBS hydrogel patch. Thus, the withdrawal of lactate via osmosis is low during exercise.

The lactate content in sweat drops after 2 hours, post-exercise. The 4 M glucose extracted ~ 17.9 nmoles of lactate (Fig. 2left), which corresponds to an average concentration of ~ 12 mM (Fig. 2right). These results suggest that 2 hours is sufficient for the lactate levels to go back to its base level (at rest). The PBS hydrogel samples ~ 11.2 nmoles of lactate, which matches well with the lactate levels at rest. This confirms that lactate in a PBS hydrogel patch during post-exercise experiments is also derived from the naturally generated sweat (same as during resting). Additionally, a higher (with respect to PBS hydrogel) lactate collection by 4 M glucose hydrogel also confirms the continued role of osmotic extraction during the post-exercise tests.

B. Correlation between Sweat and Blood Lactate

We measured the blood lactate concentration and performed a preliminary analysis of its relationship with the sweat lactate concentration under all the physiological stages (resting, during exercise, and post-exercise). The average blood lactate concentration was ~ 1.7 mM during resting and post-exercise, while it climbs up to ~ 2.5 mM during exercise. As a result, the average sweat lactate concentration stood ~5× greater than blood lactate concentration during resting and post-exercise, while ~10× greater than blood lactate concentration during exercise.

The data in Fig. 3 did not show any correlation between sweat and blood lactate levels at rest. This is expected since lactate in blood depends highly on the levels of physical exertion due to the homeostatic metabolic regulation. The medium exercise intensity experienced during these tests did not significantly change the blood lactate levels. This is expected since our operational exercise intensity is way below the lactate threshold (LT) of the human subjects. The blood lactate increases exponentially beyond LT [16]. Additionally, higher blood lactate levels can also come from pathologies and not from just physical activity [2]. The results also establish that sweat lactate during exercise is mainly derived from the stored glycogen of muscles, eventually appearing in the eccrine sweat glands. Lactate does not get released into the blood under medium exercise intensity. The post-exercise blood lactate match with the resting and exercise periods due to absence of physical exertion.

C. In-vitro Validation of Sweat Lactate Sensing Patch

Fig. 4a shows the schematic of the continuous electrochemical sweat lactate sensing platform, developed

by the prototype in Fig. 1. It consists of a hydrogel and a paper microfluidic channel, arranged in a similar configuration as shown in Fig. 1. This platform is thinner and more flexible than Fig. 1 patch since it is developed on a polyimide sheet. The hydrogel withdraws sweat from the skin surface via osmosis. The withdrawn sweat then gets wicked through the rectangular section of the paper channel, where it meets the functionalized working electrode. This leads to the generation of a current signal that varies proportionally with the amount of lactate in the sweat.

Chronoamperometry was performed to characterize the printed lactate sensors (Fig. 4b) in 1×PBS (0.15 M, pH=7.3). The current response of the lactate sensors was assessed over a lactate concentration range of 0-20 mM. An increase in the cathodic current with respect to an increase in lactic acid concentration was observed (Fig. 4b). The sensitivity and the limit of detection of the sensors were estimated to be 10 $\mu A.mM^{-1}cm^{-2}$ and 350 nM, respectively.

The selectivity of the sensor was evaluated in the presence of various levels of Na^+, K^+, uric acid (UA), ascorbic acid (AA), and glucose. As shown in Fig. 4c, the addition of interferents led to ~ 30% lesser current response as compared to the response of 5 mM lactate. The sensors also showed a satisfactory reproducibility over a wide range of 1-10 mM lactate concentration (Fig. 4d). Based on this data, the lactate concentration of artificial sweat (Pickering Laboratories Inc.) was estimated to be ~ 8 mM.

IV. CONCLUSION

We report here the results from the *in-vivo* human on-skin tests of a non-invasive sweat sampling patch. This wearable patch operates under novel sweat extraction principles and with zero electrical power. The patch uses osmosis, capillary wicking, and evaporation simultaneously for sustaining long-term inflow of sweat and biomarkers. We tested the patch for sweat lactate determination under three physiological conditions: rest, exercise, and post-exercise. Sweat lactate collection via osmosis was dominant during resting and post-exercise. Lactate collection during exercise was mainly due to the inflow of active sweat in the patch. Sweat lactate level increased under medium exercise intensities. These levels did not exhibit a correlation with blood lactate, as the exertion levels were moderate. The data suggest that the reported osmotic patch may enable lactate measurements under low or no active sweating, where the previous work in the literature would encounter difficulties in extracting and measuring sweat lactate levels.

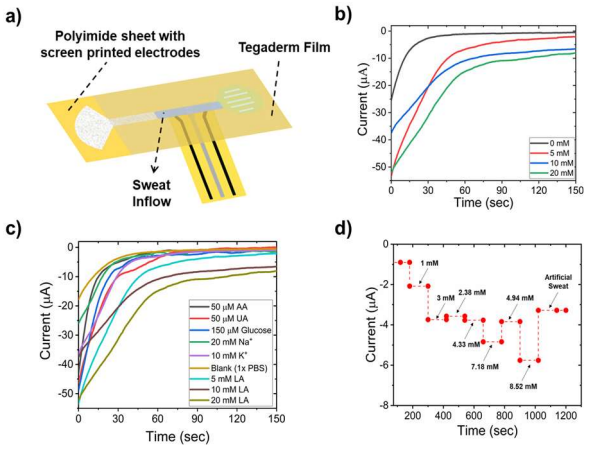

Figure 4: Characterization of the electrochemical lactate sensors. (a) Schematic of the sweat lactate sensing platform. (b) Chronoamperometry of the sensors in 1×PBS solution with varying lactic acid (0,5,10,20 mM). (c) Selectivity of the sensors in presence of common sweat biomarkers. (d) Reversibility of the sensors under varying lactic acid concentration.

We report the benchtop performance of a next generation osmotic-capillary patch with integrated continuous electrochemical sensors for sweat lactate detection. Such a wearable prototype will provide sweat sampling and managing techniques for long-term, and real-time monitoring of physical exercise and metabolic activity. Additionally, we are also working towards integrating our wearable patch with microneedles to develop a biomarker sensing platform for interstitial fluid.

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