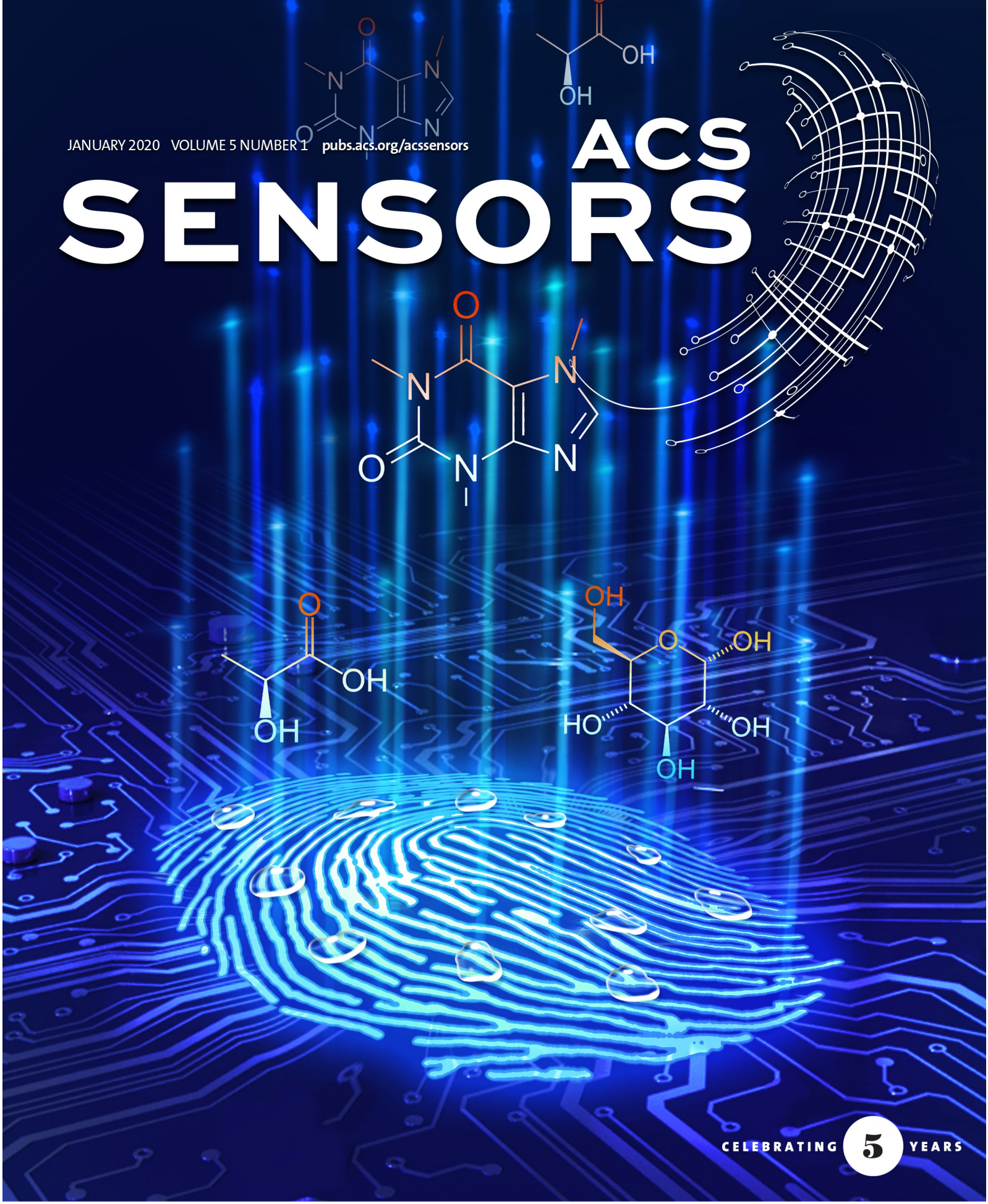
JANUARY 2020 VOLUME 5 NUMBER 1 pubs.acs.org/acssensors

ACS SENSORS



ACS Publications
Most Trusted. Most Cited. Most Read.

www.acs.org

ACS SENSORS

Article

pubs.acs.org/acssensors

Natural Perspiration Sampling and in Situ Electrochemical Analysis with Hydrogel Micropatches for User-Identifiable and Wireless Chemo/Biosensing

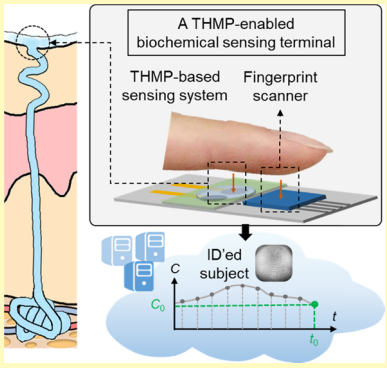
Shuyu Lin,[†] Bo Wang,[†] Yichao Zhao,[†,‡] Ryan Shih,[†,§] Xuanbing Cheng,[†,‡] Wenzhuo Yu,[†] Hannaneh Hojaiji,[†] Haisong Lin,[†] Claire Hoffman,[†,∥] Diana Ly,[†,∥] Jiawei Tan,[†,‡] Yu Chen,[⊥] Dino Di Carlo,[∥] Carlos Milla,[#] and Sam Emaminejad*[,†,∥]

[†]Department of Electrical and Computer Engineering, [‡]Department of Materials Science and Engineering, [§]David Geffen School of Medicine, [∥]Department of Bioengineering, and [⊥]Molecular Instrumentation Center, University of California, Los Angeles, California 90095, United States

[#]The Stanford Cystic Fibrosis Center, Center for Excellence in Pulmonary Biology, Stanford School of Medicine, Palo Alto, California 94305, United States

Supporting Information

ABSTRACT: Recent advances in microelectronics, microfluidics, and electrochemical sensing platforms have enabled the development of an emerging class of fully integrated personal health monitoring devices that exploit sweat to noninvasively access biomarker information. Despite such advances, effective sweat sampling remains a significant challenge for reliable biomarker analysis, with many existing methods requiring active stimulation (e.g., iontophoresis, exercise, heat). Natural perspiration offers a suitable alternative as sweat can be collected with minimal effort on the part of the user. To leverage this phenomenon, we devised a thin hydrogel micropatch (THMP), which simultaneously serves as an interface for sweat sampling and a medium for electrochemical sensing. To characterize the performance of the THMP, caffeine and lactate were selected as two representative target molecules. We demonstrated the suitability of the sampling method to track metabolic patterns, as well as to render sample-to-answer biomarker data for personal monitoring (through coupling with an electrochemical sensing system). To inform its potential application, this biomarker sampling and sensing system is incorporated within a distributed terminal-based sensing network, which uniquely capitalizes on the fingertip as a site for simultaneous biomarker data sampling and user identification.



KEYWORDS: *natural perspiration, hydrogel micropatch, biosensor, sample identification, electrochemical analysis, Internet-of-Things, lactate, caffeine*

Recent advances in miniaturized electronics, microfluidics, and electrochemical sensing platforms have paved the path for the realization of chemo/biosensing networks geared toward general population health and wellness monitoring.[1−4] To this end, a sweat-based sensing modality presents a great potential for the integration into such networks, as it provides noninvasive access to molecular-level information, enabling insight into the body's dynamic chemistry.[5−7] However, realizing effective sweat sampling remains a fundamental challenge in noninvasive chemo/biosensing.[8] Previous studies have mainly relied on either physical or chemical stimulation procedures (e.g., exercise,[9,10] heat,[11] and iontophoresis[12]) to induce sweat production in substantial volumes (~50 μL). While useful for infrequent analysis in laboratory settings, challenges remain when implementing these methods in the context of (semi)continuous measurements: exercise requires strenuous physical activity, which may not be suitable for all potential users (especially the bedridden, neonatal, or elderly populations); heat can lead to exhaustion and discomfort, which could cause noncompliance if used in a device for patients; and iontophoresis has numerous challenges related to the integration of device- and circuit-level stimulating components, which are currently being addressed.[13] It is also worth noting that the analyte concentration in sweat has been shown to be sweat gland secretory rate-dependent,[14] which, in the absence of corrective calibration mechanisms, leads to distortion in the biomarker measurements when relying on stimulation-based sweat sampling.

Given the challenges of these sampling methods, alternative methods of accessing sweat biomarkers may warrant exploration. One such alternative is naturally occurring/background thermoregulatory perspiration (hereinafter, referred to as natural perspiration). Natural-perspiration-based

Received: September 4, 2019
Accepted: November 8, 2019

DOI: 10.1021/acssensors.9b01727
ACS Sens. XXXX, XXX, XXX−XXX

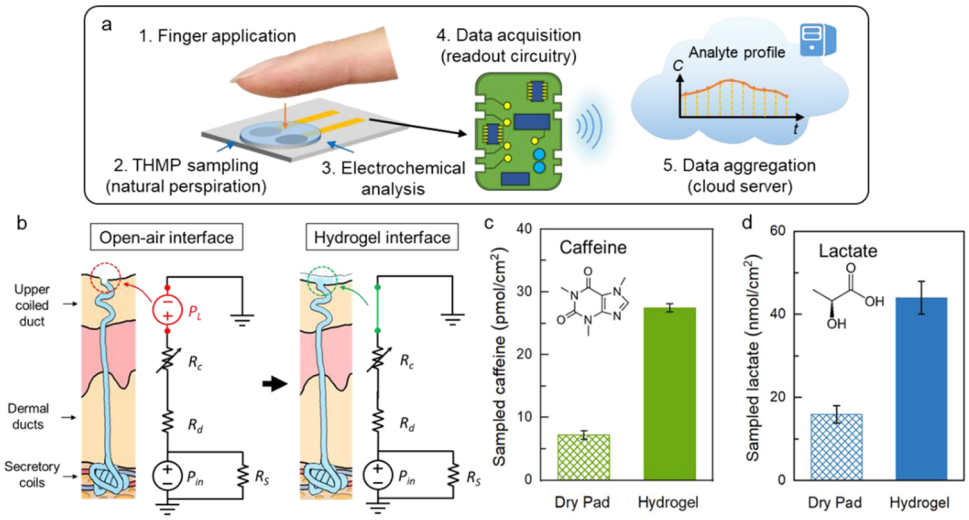


Figure 1. (a) Schematic of the THMP-based naturally perspired analyte sampling and wireless in situ electrochemical analysis on a fingertip. (b) First-order microfluidic model for natural perspiration sampling with an open-air interface and hydrogel interface. (c, d) Amount of caffeine (c) and lactate (d) sampled per unit area on the fingertips with a dry absorbent pad (can be effectively considered as an open-air sampling interface) and hydrogel interface (sampled from four different fingers of the same subject; error bars indicate standard error). Sampling events targeting caffeine were conducted 2 h after the consumption of beverages containing 150 mg caffeine.

sampling eliminates the need for active stimulation and provides a stable secretion rate (on the order of 1 nL/min per gland) within the time window of sampling.[13] This minimizes the confounding effect of sweat rate variability and leads to more accurate analysis. Furthermore, owing to its low secretion rate, natural perspiration is speculated to give adequate time for the low-membrane-permeability analytes to partition from blood to sweat, and thus sweat−blood correlation can be established for a wider range of biomarkers.[15,16] While this phenomenon has not been widely used for bioanalytical device development, academic forensic science studies have already demonstrated its utility in residual fingerprint analysis for the simultaneous rendering of biochemical and user identification information.[17] In one respect, drugs or metabolites of target compounds can be extracted from the sweat, yielding information about the individual's consumption of illicit substances with the added potential to make inferences regarding the individual's general health state.[18] Complementarily, the spatial distribution of those extracted analytes, coupled with ridge and groove patterns of the sweat left on the surface, can be exploited to produce a latent fingerprint image to uniquely identify the individual.[19]

To fully realize the potential of natural perspiration for noninvasive health monitoring applications, two fundamental challenges related to biomarker sampling and sensing must be addressed. First, instantaneously secreted sweat droplet sampling is challenging because of its low flow rate and associated volume of secretion, and such sampling requires sweat collection by accumulation over a period of time in suitable interfaces/reservoirs to facilitate effective analyte partitioning and collection from sweat glands. Second, in situ electrochemical techniques (which are commonly used for sweat analysis at the "point-of-person") require a reliable medium to envelop and protect sensing electrodes, as well as facilitate analyte transport to those electrodes for detection.

Previous work demonstrating analyte sampling interfaces for natural perspiration from the skin can generally be categorized by interface type (either dry or wet). Dry interfaces (like unwetted filter paper or the commercial PharmChek skin patches) typically require longer collection periods (on the order of days) and are thus more useful in obtaining a binary presence metric indicating if a compound has accumulated to sufficient levels for detection.[20,21] Hydrated interfaces (like wetted filter paper or hydrogels), on the other hand, are better suited for health monitoring devices and terminals aiming to study and characterize pharmacokinetic and metabolic profiles of the subjects due to their shorter sampling times and improved partitioning efficiency (greater analyte collection).[22−24] Both interface types, however, are commonly used only as reservoirs for subsequent analysis in a decoupled manner with bulky laboratory instruments (e.g., mass spectrometers). Recently, a proof-of-concept of lactate measurement with a sensor-coupled hydrogel was demonstrated for fingertip sweat sampling;[25] however, the key hydrogel sensor performance metrics (including the sensor's limit of detection (LoD), detection range, and selectivity) has not been fully characterized, and its deployment in diverse application spaces has not been demonstrated.

Here, we devise a thin hydrogel micropatch (THMP) for simultaneous natural perspiration collection and in situ electrochemical analysis (Figure 1a) and then demonstrate its utility in an integrated health monitoring setting to illustrate its enabling potential in diverse application spaces. To characterize THMP's performance, caffeine and lactate were selected as two representative target molecules. The choice of caffeine was motivated by its therapeutic potential for the treatment of airway obstruction and apnea of prematurity[26,27] and the fact that it is a xenobiotic. We particularly exploit its xenobiotic nature to perform controlled studies for determining optimal sampling conditions and demonstrating the utility of the approach for tracking dosage levels and metabolic patterns. The choice of lactate was motivated by its clinical significance in a variety of applications[28] and the fact that lactate-targeting sensors have been extensively explored for sweat analysis.[29,30] Accordingly, by coupling a custom-developed wireless enzymatic lactate sensing module with the hydrogel interface, we demonstrate a THMP-based sensing

DOI: 10.1021/acssensors.9b01727
ACS Sens. XXXX, XXX, XXX−XXX

system, which noninvasively renders sample-to-answer biomarker data from the fingertip. Furthermore, this coupled system was leveraged to demonstrate the proof-of-concept of a distributed terminal-based chemo/biosensor network, which uniquely capitalizes on the fingertip as a site for simultaneous biomarker data sampling and user identification. This terminal-based sensing modality can be embedded within the extant and future Internet-of-Thing (IoT) infrastructures for community-based health monitoring.

■ EXPERIMENTAL SECTION

Materials and Reagents. Agarose, sodium acetate buffer solution (3 M, pH = 5.2 ± 0.1), caffeine, caffeine−$^{13}C_3$ (1 mg/mL in methanol), multiwalled carbon nanotube (MWCNT, carboxylic acid-functionalized), Nafion perfluorinated resin solution (5 wt %), chloroplatinic acid hexahydrate ($H_2PtCl_6 \cdot 6H_2O$), *m*-phenylenediamine, bovine serum albumin (BSA), glutaraldehyde solution (25 wt %), poly(vinyl chloride) (PVC), tetrahydrofuran (THF), sodium L-lactate, D-glucose, uric acid (UA), ascorbic acid (AA), sodium chloride (NaCl), and potassium chloride (KCl) were purchased from Sigma-Aldrich (Missouri). 2-Propanol (IPA), water (Optima LC/MS grade), formic acid, acetonitrile (Optima LC/MS grade), and phosphate-buffered saline (PBS, pH = 7.4) were purchased from Fisher Scientific (Massachusetts). Silver−silver chloride (Ag/AgCl) ink was purchased from Ercon Incorporated (Massachusetts). Lactate oxidase (LOx) was purchased from Toyobo USA, Inc. (New York).

Molecular weight cutoff (MWCO, 10 kDa) centrifugal filters (Amicon Ultra-0.5) were purchased from Sigma-Aldrich. Double-sided tape (170 μm thick, 9474LE 300 LSE) was purchased from 3M Science (Minnesota). Poly(ethylene terephthalate) (PET, 100 μm thick) was purchased from MG Chemicals (BC, Canada). CF1 absorbent pads were purchased from GE Healthcare Bio-Sciences (Pennsylvania). Espresso containing 75 mg caffeine per shot used for caffeine testing in human subjects was purchased from Peet's Coffee (California).

THMP Fabrication. The THMPs were prepared using a layered mold assembly of a glass slide substrate, a middle layer consisting of one or more strips of double-sided tape, and a PET capping layer (Figure S1). Tape and PET layers were laser-cut to form a microfluidic chamber and access ports, respectively (Epilog Mini 24, Epilog Laser). The chamber (6 mm in diameter) was used to define the hydrogel patch size, and 1 mm inlet and outlet ports in the PET layer enabled solution injection into the chamber. Hydrogel thickness was controllably adjusted by varying the number of double-sided tape strips used to create the wall of the microfluidic chamber. Hydrogel (containing 2% agarose) was then prepared by dissolving agarose powder in a 0.01 M acetate buffer (diluted from a 3 M stock solution, pH = 5, similar to sweat[31]) followed by placing the resulting mixture in an 80 °C water bath. After agarose was completely dissolved, the solution was immediately injected into the preassembled mold (heated to 80 °C on a hot plate). Following sufficient cooling, the fabricated hydrogel patch was obtained by peeling the tape and PET layers from the glass slide. Patch volumes were calculated from weight measurements assuming a density of 1 g/mL.

Natural Perspiration Sampling. For natural perspiration sampling intended for caffeine analysis, participants with a regular caffeine intake (e.g., routine or daily consumption of caffeinated beverages) were excluded to avoid confounding factors (e.g., caffeine accumulation in body),[32] and all of the participants were required to abstain from the consumption of food, beverages, and drugs containing caffeine for 24 h prior to the experiment. The skin sampling regions of interest were washed with hand soap sheets (Travelon, Illinois) and deionized (DI) water followed by wiping with Kimwipes containing 70% IPA. The efficiency of the adopted cleaning protocol is demonstrated in Figure S2. A THMP or absorbent pad was placed on the skin for 5 min for sweat sampling, and a polyethylene plastic film was used to cover the device to avoid evaporation. Given that the natural perspiration rate, on the high end, is on the order of 0.4 mg/(cm^2 min)[33] (which is much higher than the rate of observed transepithelial water loss[34]), the incremental volume contribution of natural perspiration to the THMP is less than 1 μL. As the original THMP volume is ∼8 μL, the volumetric contribution by natural perspiration results in dilution by a factor of ∼10, allowing to establish a linear relationship between the collected analyte amount and the corresponding analyte concentration in perspiration.

Lactate Quantification with Lab Instrument. After natural perspiration sampling, the THMP or absorbent pad was placed into an Eppendorf tube containing 100 μL of acetate buffer (0.01 M, pH = 5), and the tube was sonicated for 5 min at room temperature for the extraction of the target molecules. The lactate concentration in the tube was quantified directly with the YSI 2900 analyzer (YSI Incorporated, Ohio) in duplicate, and the average value was used. The amount of lactate sampled per unit area was determined from the concentrations in the extraction buffer, extraction buffer volume, and the area of the THMP or absorbent pad.

Caffeine Quantification with Liquid Chromatography−Tandem Mass Spectrometry (LC−MS/MS). The amount of caffeine sampled with the THMP or absorbent pad was measured by LC−MS/MS with multiple reaction monitoring (MRM) technique (Figure S2). ^{13}C isotopically labeled caffeine (caffeine−$^{13}C_3$) was used as the internal standard (IS). For calibration, different concentrations of caffeine were spiked into water (Optima LC/MS grade) to make standard solutions of 0.0, 1.0, 2.0, 5.0, 10.0, 50.0, 100.0, and 200.0 nM. For the analysis of real samples, the perspiration-sampled THMP or absorbent pad was placed into an Eppendorf tube containing 200 μL of acetate buffer (0.01 M, pH = 5), and the tube was sonicated for 5 min at room temperature for target molecule extraction. A molecular-weight cutoff (MWCO) filtering was used to remove any particulate matter from the sample. The standard solution or real sample extraction of 150 μL was mixed with 1.5 μL of 10.0 μM IS (to reach a final IS concentration of 0.1 μM), loaded into a 10 kDa MWCO filter, and centrifuged at 14 000*g* for 10 min. The low-molecular-weight filtrate was then transferred into an autosampler vial for caffeine quantification.

An Agilent 1200 series HPLC (Agilent Technologies, California) equipped with a HTS PAL autosampler (CTC Analytics, Minnesota) was coupled to an API 4000 triple quadrupole mass spectrometer (Sciex, ON, Canada) for MRM experiments. A Zorbax 300 SB-C18 column (0.5 ID × 150 mm L, 5 μm, Agilent Technologies) was used for separation. Solvent A was water with 0.1% formic acid, and solvent B was acetonitrile with 0.1% formic acid. The flow rate was 400 μL/min with the following gradient: 5% B (0−0.5 min), from 5 to 90% B (0.5−5.5 min), 90% B (5.5−8.5 min), from 90 to 5% B (8.5−9.0 min), 5% B (9.0−11.0 min). Sample vials were maintained at 4 °C in the autosampler tray. A sample of 20 μL was loaded onto the column each time.

The instrument was operated in an MRM mode with the following *m*/*z* transitions: 195.2 → 138.1 for caffeine and 198.1 → 140.1 for caffeine−$^{13}C_3$. The declustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP) were optimized at 71, 10, 27, and 8 V for caffeine, and 86, 10, 27, and 8 V for caffeine−$^{13}C_3$, respectively. Ion spray voltage and temperature were 5500 V and 400 °C, respectively. The collision gas, curtain gas, and ion source gas 1 and 2 were set at 4, 30, 30, and 50 psi, respectively.

Lactate Sensing Module Fabrication. The lactate sensing module was fabricated on a pair of gold (Au) electrodes (diameter: 2.4 mm, 20 nm Cr/100 nm Au) deposited and patterned on the PET substrate. The reference electrode was fabricated by drop-casting the Ag/AgCl ink directly on the Au electrode followed by baking at 80 °C for 10 min. To fabricate the working electrode, 1.15 μL of the MWCNT solution (2 mg/mL in a 5 wt % Nafion solution) was drop-cast onto the Au electrode and dried at an ambient environment. Platinum nanoparticles (PtNPs) were then electrochemically deposited onto the MWCNT-modified Au electrode in the PtNP deposition solution (2.5 mM H_2PtCl_6 and 1.5 mM formic acid in DI water) using cyclic voltammetry (0−−1.0 V vs Ag/AgCl, 20 cycles, 50 mV/s). The poly-*m*-phenylenediamine (PPD) layer was subsequently

DOI: 10.1021/acssensors.9b01727
ACS Sens. XXXX, XXX, XXX−XXX

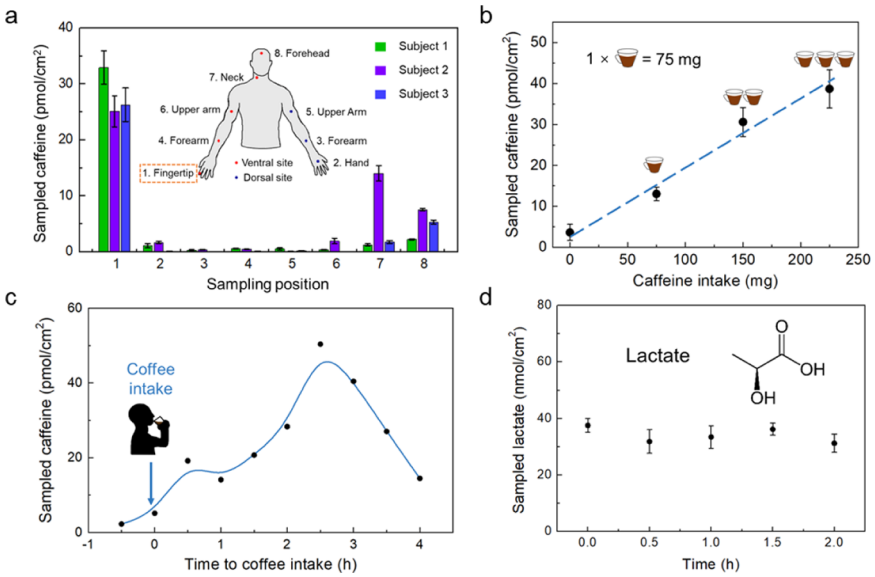


Figure 2. Characterization of naturally perspired analyte sample with THMP. (a) Amount of caffeine sampled per unit area on different body sites (three subjects with three trials at each position; sampled 2 h after the consumption of a beverage containing 150 mg of caffeine; error bars indicate standard error). (b) Amount of caffeine sampled per unit area on the fingertips of the same subject with different caffeine dosages (sampled from three different fingers, 2 h after the consumption of beverages with the annotated caffeine content; error bars indicate standard error). (c) Amount of caffeine sampled per unit area on the fingertips of a single subject at 30 min intervals before and after the consumption of a beverage containing 150 mg of caffeine. (d) Amount of lactate sampled per unit area on the fingertips at 30 min intervals (from a sedentary subject, sampled from three different fingers; error bars indicate standard error).

electrochemically deposited onto the Au/MWCNT/PtNP electrode with the *m*-phenylenediamine solution (5 mM in PBS) by applying 0.85 V (vs Ag/AgCl) for 120 s. The modified working electrode was then washed with DI water and dried. To immobilize LOx, BSA was used as a stabilizer and glutaraldehyde was used as a cross-linker. Specifically, 500 μL of the BSA solution (10 mg/mL in PBS) was first mixed with 4 μL of glutaraldehyde solution (25 wt %), followed by mixing with the lactate oxidase solution (10 mg/mL in PBS) at a 1:1 ratio (v/v). The enzyme mixture (1.15 μL) was drop-cast onto the Au/MWCNT/PtNP/PPD electrode and dried at room temperature. Then, the sensor was dip-coated with the PVC solution (3 wt % in THF) twice and dried at 4 °C in a dark environment overnight. The fabricated sensors were stored at 4 °C.

Lactate Sensing Module Characterization and Measurement. The thickness of each layer of the lactate sensor was measured by the Dektak 6M profilometer. To characterize the electrochemical performance of the developed lactate sensing module, constant potential amperometric measurements were conducted at +0.5 V vs Ag/AgCl. By drop-casting the lactate solution (in PBS, pH = 7.4) of different concentration levels onto the corresponding sensors, calibration plots were obtained, which were utilized to determine the performance of the developed sensing interface. The limit of detection (LoD) for each amperometric sensing interface was calculated as LoD = 3 × SD/slope, where SD is the standard deviation of the baseline noise in the PBS, and the slope is defined as the amperometric current change vs lactate concentration change. Sensitivity is calculated as the slope divided by the sensor's surface area. To characterize the THMP-based sensing module, the THMPs were soaked in the analyte solution (in PBS, pH = 7.4) for at least 10 min and then transferred onto the sensor surface (while ensuring that minimal lactate solution residue was present on the surface of the THMP). Corresponding amperometric measurements were conducted after placing the presoaked THMP onto the sensor. Lactate tracking on the fingertips was performed by instructing the subject to press the index fingertip onto the THMP for 5 min, followed by taking an amperometric measurement upon the removal of the fingertip.

Readout Circuit Module for THMP-Based Wireless Sensing System. The wireless amperometric measurement was realized with a miniaturized readout circuitry similar to our previous effort..[13] The circuitry includes an ultralow-power microcontroller unit (MCU, STM8L-UFQFN20, STMicroelectronics, California), which is interfaced with an onboard Bluetooth transceiver to wirelessly and bilaterally communicate user commands and the sensor's output current in real time with a custom-developed smartphone application. The MCU controls the developed analog signal acquisition and conditioning circuits interfacing the THMP-based sensing module. An onboard potentiostat chip (LMP91000, Texas Instruments, Texas) was programmed to apply +0.5 V across the working and the reference electrodes and set to convert the acquired current signal to voltage values with the aid of its internal transimpedance amplifier. The corresponding output voltage was filtered by a fifth-order low-pass filter module (MAX7422 chip, cutoff frequency: 1 Hz, Maxim Integrated, California) and converted into the digital domain by a 12-bit analog-to-digital unit (built-in in the MCU). A compact rechargeable lithium-ion polymer battery with a nominal voltage of 3.7 V and a capacity on the order of 100 mAh was used to power the readout board.

User Identification Module. The fingerprints of the users were collected and identified with a commercial fingerprint scanner module (TTL GT-511C1R, SparkFun Electronics, Colorado), which bilaterally communicates with a microcontroller (Atmega8) or the provided software. The fingerprint templates of all of the users were stored in the fingerprint scanner module before identification tests.

Institutional Review Board (IRB) Approval for Human Subject Testing. The conducted human subject experiments were performed in compliance with the protocols that have been approved by the IRB at the University of California, Los Angeles (IRB #17-000170). All subjects gave written informed consent before participation in the study.

RESULTS AND DISCUSSION

Naturally Perspired Analyte Sampling. To set up a framework for natural-perspiration-based analyte sampling, we expand upon the first-order microfluidic models of eccrine sweat glands presented in the previous studies..[16] As shown in

DOI: 10.1021/acssensors.9b01727
ACS Sens. XXXX, XXX, XXX−XXX

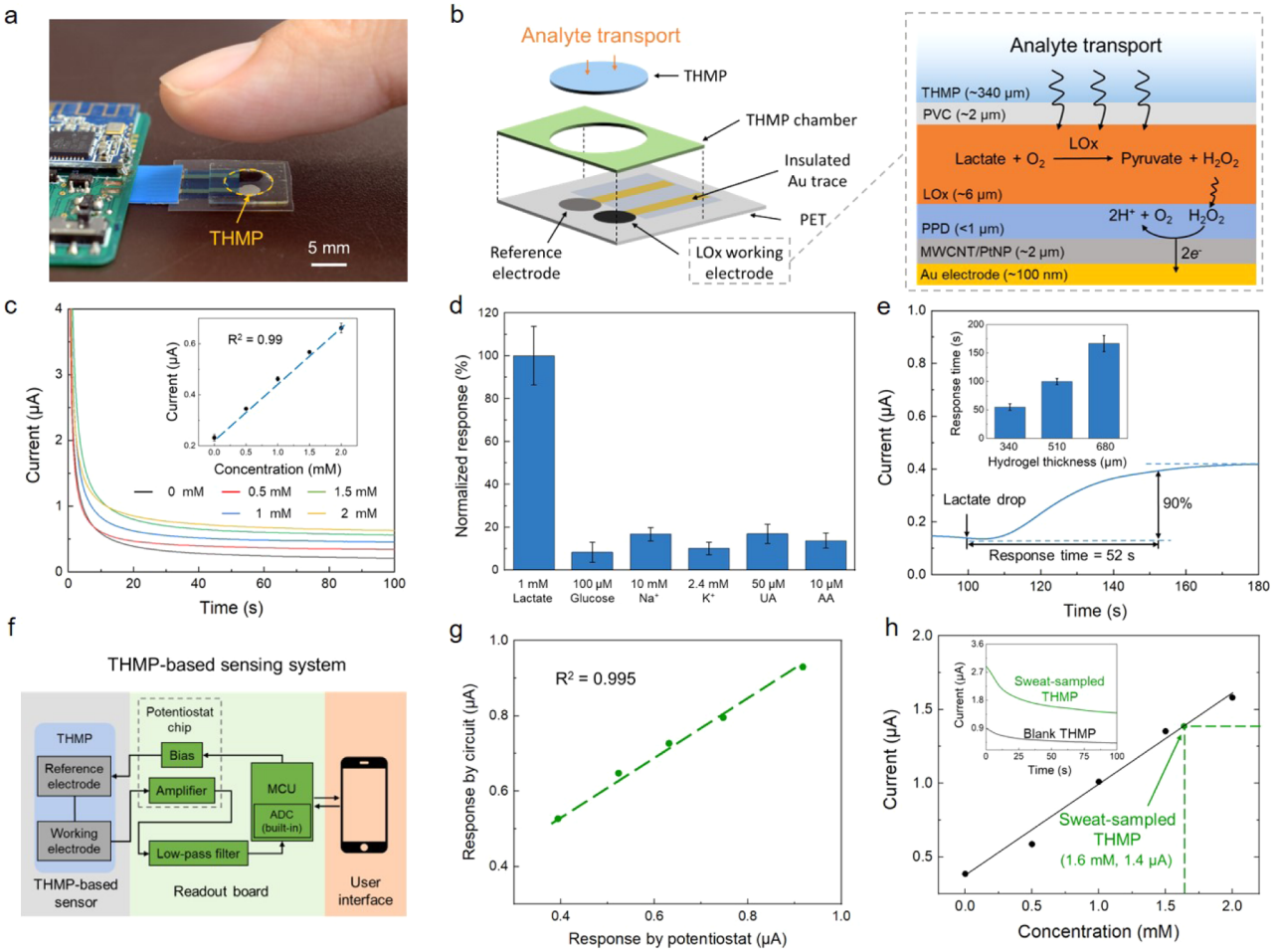


Figure 3. (a) Image of a one-touch THMP-based wireless electrochemical sensing system (sampling from a fingertip). (b) Schematic of the THMP-based lactate sensing module. Zoomed view (right) shows the structure of the LOx working electrode (with the approximate thickness of each layer annotated). (c) Measured amperometric responses of THMP-based lactate sensing module upon the placement of hydrogels presoaked in solutions with different lactate concentration levels. The inset shows the corresponding calibration curve (error bars indicate standard error, measured with three different THMPs). (d) Selectivity study of THMP-based lactate sensing module. The tests were performed separately, with THMPs presoaked in solutions containing different interfering species. Measured amperometric responses were normalized to a 1 mM lactate readout (error bars indicate standard error, $n = 3$). (e) Dynamic THMP-based amperometric response to 1 μL of a 10 mM sodium lactate concentrated solution, drop-cast onto a 340 μm-thick blank hydrogel. The inset shows the response time vs hydrogel thickness (error bars indicate standard error, $n = 3$). (f) System-level block diagram of the THMP-based wireless sensing system (interfaced with a readout board and a mobile application). (g) Amperometric response of a THMP-based lactate sensor measured by the developed readout circuitry vs potentiostat. (h) Representative measurement (1.4 μA) derived from the placement of the fingertip on the integrated sensing system, determining the sampled lactate concentration to be 1.6 mM (upon postcalibration). The nset shows the raw recording of the corresponding measurement, as well as the recording for the case of a blank THMP (included for comparison).

Figure 1b, P_{in} is the varying hydrostatic−osmotic pressure (originating from the accumulation of Na^+ and Cl^- ions in the secretory lumen[35]), which pumps sweat from the glands to the pores on the skin surface, forming sweat droplets. R_c, R_d, and R_s are the fluidic resistances of the upper coiled duct, the dermal duct, and the secretory coil, respectively. The surface-tension-induced Laplace pressure, P_L, acts to oppose sweat secretion, where the surface tension corresponds to that of a sweat droplet with a finite radius of curvature forming on the surface of the skin. For natural perspiration to occur, P_{in} must be increased beyond P_L. Once perspiration has occurred, P_{in} will be reduced (due to the loss of the accumulated Na^+ and Cl^- ions upon secretion) and must rise above the P_L threshold again to induce the next cycle of the sweat secretion, giving rise to the pulsatile behavior of natural perspiration. This behavior is consistent with the previous observations[16] and our own examination of natural perspiration (Supporting Information Movie 1, a video recording of a subject's fingertip), where the naturally perspired droplets were generated and then evaporated.

To eliminate the P_L pressure barrier, a hydrogel-based collection interface is devised. By creating a continuous hydrophilic fluidic path connecting the secretory coil and sampling reservoir, the hydrogel effectively reduces P_L to zero (Figure 1b, right). In this way, even a small P_{in} buildup will result in sweat secretion that can be utilized for analyte collection by the hydrogel. In addition, due to its aqueous nature, the hydrogel interface also serves to facilitate the diffusion of analytes from sweat to the reservoir. These two features render the hydrogel an effective collection reservoir for capturing analyte flux. To illustrate the analyte collection efficacy of the devised THMP, perspiration sampling experiments targeting caffeine and lactate (as two representative molecules) were performed. The caffeine sampling experi-

DOI: 10.1021/acssensors.9b01727
ACS Sens. XXXX, XXX, XXX−XXX

ments were performed 2 h after the consumption of caffeinated beverages. The analyte collection capability of the THMP was compared with that of the dry absorbent pad (which can be effectively considered as an open-air sampling interface in the described model, laser-cut to be the same size as the THMP). For each target, the two sampling media were placed side by side on a subject's fingertip. After sampling, the two media were immersed in acetate buffer solutions for analyte extraction followed by quantification. Caffeine concentration levels were measured by LC−MS/MS (similar to previous effort,;[36,37] calibration shown in Figure S3), and lactate concentration levels were measured by a YSI analyzer. The analyte sampling characterization results for both molecules indicated that the hydrogel interface sampled approximately three times as much analytes as the dry pad interface (Figure 1c,d), which is in agreement with the described first-order model and informs the enhanced analyte collection capability of the THMP.

To further validate the suitability of THMP for analyte sampling, it was deployed on eight different body sites for caffeine sampling on three subjects. Figure 2a shows that the hydrogel micropatch was able to collect detectable (by LC−MS/MS) levels of xenobiotics for most of the sampling points. In particular, for all of the subjects, the highest amounts of analytes (per unit area) were collected from the

fingertips among the investigated body sites. This observation may be attributed to the fact that the fingertips have the highest density of eccrine sweat glands[38] and/or that blood capillaries lie in close proximity to the fingertip's sweat glands.[39] From this investigation, the fingertips were determined to be the optimal site for sampling due to their higher analyte yield, and thus this site was used to conduct the subsequent analyte collection characterization experiments.

To illustrate the utility of the THMP for natural perspiration sampling, we demonstrated its capability to track different caffeine intake dosage levels in a subject. Accordingly, the subject was instructed to consume caffeine-based beverages with four different caffeine amounts (0, 75, 150, and 225 mg), and at least 24 h of washout period was included in between to avoid the carryover effect. THMP-based sampling was performed 2 h after each caffeine intake. As shown in Figure 2b, the quantification results indicate that the amount of caffeine sampled in the hydrogel increased linearly with increasing dosage, which, in turn, illustrates the reliability of the THMP-based sampling method. Furthermore, we employed THMP to characterize the temporal metabolic pattern of caffeine (as a xenobiotic). Accordingly, THMP-based sampling on a subject's fingertip was performed at 30 min intervals before and after the consumption of a beverage containing 150 mg of caffeine. As shown in Figure 2c, for this subject, the sampled caffeine peaked around 2.5 h after caffeine intake followed by a steady decline. Similar experiments with 1 h sampling intervals were also conducted for three additional subjects, where the captured caffeine metabolic patterns (Figure S4) were consistent with that observed in the 30 min interval study. On a broader level, the results also inform the feasibility of utilizing this approach to construct the pharmacokinetic profiles of xenobiotic compounds including pharmaceutical drugs. As a complementary test, to evaluate the THMP's ability to render an endogenous compound's temporal profile, THMP-based fingertip sampling was performed targeting lactate. Accordingly, one subject was monitored at 30 min intervals in a sedentary state (where no significant change in sweat lactate level was expected). The results indicate that the sampled lactate by the THMP was relatively stable over the duration of the test (Figure 2d), validating the consistency of the analyte sampling capability of the THMP.

THMP-Based Wireless Electrochemical Sensing System. With the THMP established as an effective sampling reservoir, we next investigated its suitability as an electrochemical sensing medium. In that regard, one may need to note that the electrochemical sensor response (which is proportional to the target concentration in the hydrogel) must be measured in relation to the hydrogel thickness to infer the flux of the target molecules into the hydrogel reservoir. Here, analyte sampling was performed with hydrogel micropatches of three different thicknesses (340, 510, and 680 μm, placed side by side on a subject's fingertip). Lactate and caffeine were targeted and quantified with the standard lab instruments. The results show that for the tested sampling time (5 min), the amount of target analytes sampled was relatively constant among different thicknesses, implying the presence of a higher analyte concentration in a thinner hydrogel (Figure S5), which, in turn, relaxes the requirements for sensor sensitivity and limit of detection.

Based on these findings, the thinnest THMP (340 μm thick) was subsequently used to couple with an electrochemical sensing system to noninvasively render sample-to-answer biomarker data. As a proof-of-concept, we targeted lactate with a custom-developed enzymatic sensing system (Figure 3a). The lactate sensing module was composed of a THMP affixed upon a flexible PET substrate patterned with functionalized Au electrodes (Figure 3b). The electrodes were functionalized to implement a mediator-free electroenzymatic sensing interface.[40,41] The structure of the working electrode was composed of (1) a PVC layer interfacing the THMP, as a diffusion-limiting membrane (to enhance the sensor dynamic range); (2) a LOx layer, which generates hydrogen peroxide (H_2O_2)

in proportion to the lactate concentration level in the THMP; (3) a PPD layer, which mitigates the confounding effect of electroactive species present in the sampled perspiration (e.g., UA and AA); and (4) an MWCNT/PtNP layer, which enhances the sensor sensitivity (leveraging the nanostructure's high catalytic activity and large surface area). A linear response of the developed sensing interface toward lactate was observed over a range of 0−4 mM with the sensitivity of 1.88 ± 0.24 μA/(mM cm^2) and the LoD of 0.12 ± 0.02 mM (Figure S6).

Calibration of this THMP-based sensing module was performed with THMPs presoaked with lactate solutions (with concentration levels in the range of 0−2 mM), displaying a linear response with high repeatability (Figure 3c) and indicating the suitability of the THMP interface for electrochemical sensing applications. Expanding on these findings, selectivity tests were performed to verify the biofluid (as a complex matrix) sensing capability of the THMP−sensor-coupled interface. To this end, the THMP-based lactate sensing module was challenged against a diverse panel of interfering species at physiologically relevant concentration levels (including electrolytes, electroactive species, and other small molecules). Figure 3d shows that the presence of each nontargeted analyte has minimal response compared to that of lactate. Furthermore, the sensor shows a negligible response to the sequential introduction of those nontargeted analytes (Figure S7). As shown in Figure S8, the sensor's biofluid

DOI: 10.1021/acssensors.9b01727
ACS Sens. XXXX, XXX, XXX−XXX

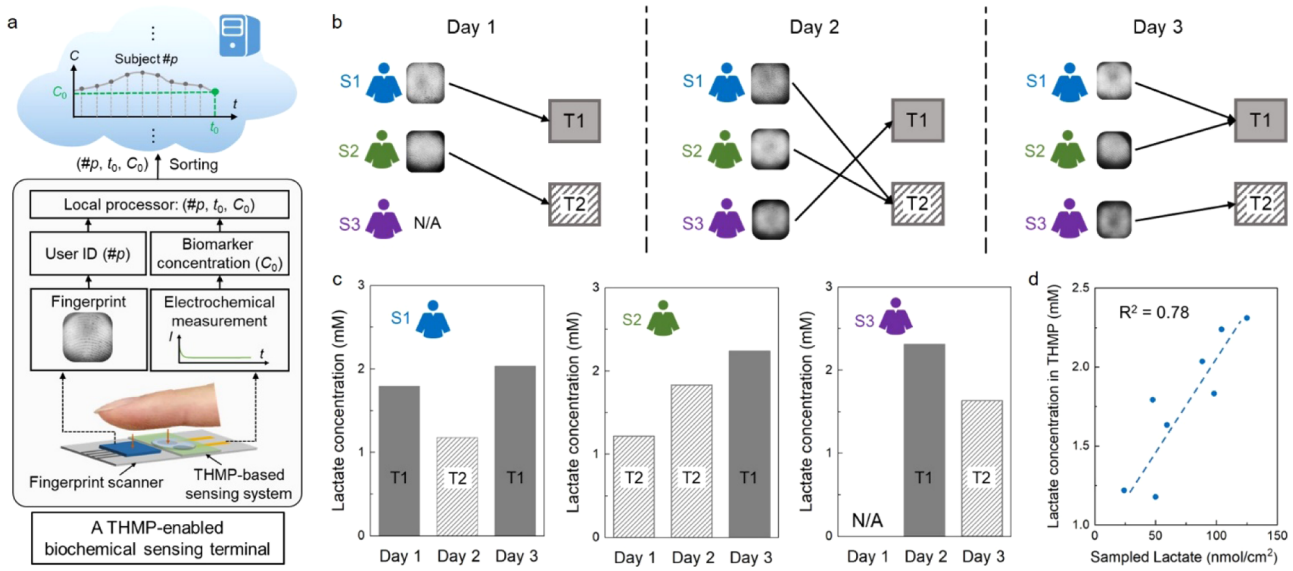


Figure 4. Terminal-based sensing network. (a) Schematic of the structure and function of a one-touch THMP-enabled biochemical sensing and user identification terminal. (b) Tracking sweat lactate among a group of three individuals (S1−S3) using two cloud-connected terminals (T1 and T2). Imaged fingerprints of each user entry are included, and each arrow indicates the terminal used by each subject (N/A indicates no entry by the subject). Photograph (right) shows a representative terminal. (c) Corresponding user-identified and sorted sweat lactate profiles of the users, rendered by the devised terminals. (d) Comparison of the in situ measured lactate concentration levels in THMP vs the corresponding amount of the sampled lactate measured by a lab instrument (YSI analyzer).

sample quantification capability was further validated by comparing the estimated lactate concentration from the developed sensor with that analyzed by a standard lab instrument (YSI analyzer) toward a 10× diluted sweat (mimicking the dilution of the sampled perspiration within the THMP).

As an additional sensing performance parameter relevant to the context at hand, the response time of the sensing module was characterized, which is dependent on the analyte diffusion dynamic (assuming operation in the diffusion-limited regime, diffusion time $\tau \propto H^2/D$, where H is the diffusion length, or the thickness of the THMP, and D is the analyte diffusion coefficient inside the hydrogel). Accordingly, a hydrogel was mounted onto the sensor from $t = 0$ to 100 s, after which 1 μL of the concentrated sodium lactate (10 mM) sample was drop-cast onto hydrogel to mimic the natural perspiration phenomenon. Hydrogels with different thicknesses 340, 510, and 680 μm were utilized to investigate the influence of hydrogel thickness to the response time of the sensing module. The sensing module's current response was monitored continuously, and the response time was defined and recorded at the time corresponding to that where the sensor current response reached 90% of its maximum current change (representative recording of the 340 μm-thick hydrogel case is shown in Figure 3e). The measurements demonstrated that thinner hydrogels exhibited shorter response times, indicating the superior dynamic performance of a thin hydrogel (Figure 3e, inset). Considering the disposable nature of the THMP-based sensing module, the lifetime of the lactate sensor and the shelf life of THMP and sensor were investigated. Accordingly, Figure S9 shows the continuous operation of the lactate sensor for 4 h, demonstrating a sufficiently long lifetime of the developed sensor. The shelf life of THMP and the lactate sensor was characterized separately. To store THMP, a hydrophobic plastic-based package was designed (Figure S10a, inset), which minimizes the water evaporation over time. As shown in Figure S10a, less than a 10% weight loss of THMP was observed after the storage of THMP within this package for longer than 120 h at room temperature. In addition, Figure S10b,c shows that the representative characterized lactate sensors maintain their responsiveness after 5 days of storage (at 4 °C). Future efforts with the particular focus on preserving the enzyme activity can be geared toward improving the shelf life.

To realize a fully integrated and self-sufficient biomarker sampling and sensing system, the sensing module was interfaced with a custom-developed Bluetooth-enabled readout board, which implements seamless and in situ signal acquisition, processing, and wireless transmission to a dashboard (e.g., a mobile application; Figure 3f). At its core, the board (Figure S11) functions as a potentiostat circuit, consisting of biasing, amplification, and filtering stages, controlled by an MCU. Prior to its deployment for on-body biomarker measurements, the board's signal acquisition and processing functionalities were validated by comparing its readings against a commercial potentiostat for a range of lactate concentration levels. Figure 3g shows a high level of linearity between the board and potentiostat readouts. The fully integrated sensing system was further validated by demonstrating its ability to estimate the lactate level, sampled directly from a subject's fingertip. Figure 3h demonstrates a representative readout and the corresponding calibrated lactate concentration level (raw recording is shown in the inset and is compared to the readout of a blank THMP).

Terminal-Based Sensing Network. The noninvasive and passive biomarker sampling and sensing nature of the THMP-based wireless sensing system make it suitable for general population monitoring, presenting a great potential for incorporation within the IoT infrastructure. To this end, we demonstrated a proof-of-concept for a distributed biochemical sensing network, which can be modeled as an array of k-cloud-connected terminals (each employing a THMP-based sensing

DOI: 10.1021/acssensors.9b01727
ACS Sens. XXXX, XXX, XXX−XXX

system), shared by n users. The terminals exploit the fingertip as the sampling site, which uniquely allows for the simultaneous collection of the user identification information (from the fingerprint) and sweat biomarker information (from natural perspiration). The capture of fingerprint information allows individuals to take their biomarker reading with shared terminals at different time points via exploiting the one-touch biochemical sensing/user identification capability. In that regard, user identification along with biomarker sensing eliminates an extra step for the user intervention, allowing for the convenient use of terminals for biomarker sampling.

As shown in Figure 4a, each terminal is composed of (1) an identification module, which, at its core, employs a commercial fingerprint scanner module and is configured to obtain the fingerprint information from the top-half of the fingertip and identify the corresponding user by comparing the imaged fingerprint to a database of fingerprints, and (2) a THMP-based sensing system that captures the concentration of target molecules from the lower half of the fingertip. Upon one-touch information sampling, the biomarker information is paired with the imaged fingerprint and linked to the user's identity at the terminal level. Then, the user-identified and time-stamped biomarker information is subsequently transmitted, with the aid of a local processor, to cloud servers online, for information sorting and updating the user's record. To assess the one-to-one mapping capability of the identification module, the user identification based on the top-half of the fingertip (Figure S12) was preliminarily characterized by creating a database of 20 index finger fingerprints from individual subjects and verifying that the subsequently introduced partial fingerprints were correctly identified (provided by the same group of subjects). This result is aligned with the previous studies demonstrating the robustness and accuracy in subject identification based on partial fingerprint imaging.[42]

Here, the proof-of-concept for the envisioned distributed biochemical sensing network was implemented to track lactate (using the readily developed and described THMP-based lactate sensing system) among a group of three individuals (n = 3, two adult males and one female) using two cloud-connected terminals (via a cellular module, k = 2). Over a period of 3 days, these individuals were instructed to use the terminal, depending on their convenience and availability. A database of their index finger fingerprints was created and loaded onto the fingerprint scanner module of each of the terminals to allow for tracking the user entries. The recorded entries confirmed that Subjects 1 and 2 provided one sample per day, and Subject 3 only used the terminal on the second and third day (Figure 4b).

For each entry, the electrochemically generated sensor response was postcalibrated, user-identified, time-stamped, and then transmitted to a custom-developed Google Cloud platform. The uploaded data are sorted according to the existing user ID database, yielding the sweat lactate profiles of each individual (Figure 4c). To validate the lactate readings, analytes sampled in the THMP were extracted into acetate buffer solutions after each electrochemical measurement, and the lactate concentration levels were quantified by the commercial lab instrument (YSI analyzer). Quantification by the YSI analyzer before and after amperometric measurement indicated that the electrochemical measurement process had minimal influence on the lactate present (Figure S13). Figure 4d shows that the calibrated concentration levels obtained from the THMP-based sensing system and the corresponding amount of the sampled lactate quantified by the lab instrument reading are correlated (R^2 = 0.78). The results indicate that the devised sensing terminal can capture the trend of the sampled lactate amount variation. It is also worth noting that the nonideal characteristics in the measurements (e.g., finite x/y-axis intercept, inconsistent relative change between some of the pairs of measurements) may be related to the fact that (1) the measurement of YSI in the low-concentration regime is prone to error and (2) a small portion of the sampled analyte may stay on the THMP surface and may not diffuse into the bulk hydrogel. To this end, system-level calibration can be incorporated to correct such nonideal characteristics.

On a broader level, the subject-independent and terminal-based nature of the demonstrated network allows for the integration of other sensors and automated back-end processing modules (including sample preparation and calibration units) to target a wide panel of biomarkers.

CONCLUSIONS AND OUTLOOK

In conclusion, a thin hydrogel micropatch was devised for simultaneous natural perspiration collection and in situ electrochemical analysis. A first-order fluidic model was used to describe the enhanced analyte collection capability of the hydrogel, a property that was verified through caffeine and lactate sampling. Informed by the biomarker sampling characterization results across different body locations, the fingertip was determined as the optimal site for natural perspiration sampling, which was subsequently used to demonstrate the successful tracking of the dosage level and metabolic pattern of the caffeine intake among different subjects. The suitability of THMP as an electrochemical sensing medium was characterized by augmenting the THMP with a wireless enzymatic lactate sensing module, for which excellent reproducibility, selectivity, and response time ~50 s were demonstrated. These results inform the utility of natural-perspiration-based biomarker analysis, which can create new directions for noninvasive chemo/biosensing. To reinforce this point, we demonstrated the proof-of-concept for an unprecedented distributed terminal-based bio/chemical sensor network, which uniquely capitalizes on the fingertip as a site for simultaneous biomarker data sampling (via natural perspiration) and user identification (via fingerprint).

Future work will focus on utilizing and building upon the presented technology to characterize and address relevant confounding factors (that, at the current stage, remain underexplored, because of the lack of suitable technologies to perform such studies) toward establishing the physiologically meaningful interpretation of the biomarker readings. In that regard, future engineering efforts will involve the incorporation of a perspiration rate sensing interface to normalize the THMP-based biomarker readings in relation to the perspiration rate, mitigating the confounding effects of inter/intrasubject perspiration rate variability (assuming a direct measure of the analyte concentration in sweat is needed). Also, a standard skin surface cleaning component in the chemo/biosensing terminal will be devised to achieve autonomous operation and to minimize skin contamination. Furthermore, large-scale clinical investigations will be performed to systematically characterize the contribution of relevant underlying biological sources (e.g., natural perspiration, sweat gland/skin metabolism, transepithelial water loss) and understand the corresponding analyte partitioning pathways, to establish the physiological significance of the

DOI: 10.1021/acssensors.9b01727
ACS Sens. XXXX, XXX, XXX−XXX

biomarker readings (e.g., correlation with blood concentration levels) for the context at hand. The joint engineering and clinical efforts will converge to realize a scalable network with fully automated bioanalytical capabilities to render physiologically relevant information. The presented noninvasive chemo/biosensing network can be positioned within the IoT infrastructure, widening the accessibility of personalized health monitoring for the general population.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.9b01727.

Schematic of THMP array fabrication; sampled lactate with different collection procedure; LC−MS/MS for caffeine quantification; caffeine sampling from three subjects at 1 h intervals; relationship of hydrogel thickness and amount of analyte sampled, and the corresponding analyte concentration within the hydrogel; calibration curves of three representative lactate sensors; selectivity of lactate sensor; validation of diluted sweat measurement; lifetime of lactate sensor; long-time storage of THMP-based sensing module; readout circuitry used in THMP-based sensing system; fingerprint information acquisition from the top-half of the fingertip; influence of electrochemical measurement to the lactate concentration in THMP (PDF)

Natural perspiration on the fingertip of a subject (MOV)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: emaminejad@ucla.edu.

ORCID

Shuyu Lin: 0000-0002-5248-6124

Bo Wang: 0000-0002-3986-3415

Dino Di Carlo: 0000-0003-3942-4284

Author Contributions

S.L. and B.W. contributed equally. S.L., B.W., and S.E. conceived the idea and designed the experiments; S.L. led the experiments (with assistance from B.W., Y.Z., R.S., X.C., W.Y., H.H., H.L., C.H., D.L., J.T., Y.C., D.D.C., C.M., and S.E.); Y.Z. and X.C. led the sensor fabrication; W.Y. and H.H. led PCB and system design; S.L., B.W., R.S., Y.Z., X.C., W.Y., H.H., H.L., Y.C., D.D.C, C.M., and S.E. contributed to data analysis and interpretation; S.L., B.W., R.S., X.C., W.Y., H.H., Y.C., D.D.C., C.M., and S.E. drafted the manuscript, and all authors provided feedback. S.E. supervised the study.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the S.E.'s startup package provided by the UCLA Henry Samueli School of Engineering and Applied Sciences. Components of research are supported by the National Science Foundation (Award #1722972), Stanford Genome Technology Center Distinguished Young Investigator Award (in partnership with Intermountain Healthcare), PhRMA Foundation (Research Starter Grant in Translational Medicine and Therapeutics), and the funding secured by the Preservation of the Force and Family Program at US Special Operations Command (executed as a subaward issued to the University of California at Los Angeles by the Henry M. Jackson Foundation under a cooperative agreement with the Uniformed Services University). The authors appreciate UCLA nanoelectronics research facility (NRF) for their help in device fabrication/characterization. The authors also thank Christopher Yeung, Zhaoqing Wang, Kayla Choi, Sina Moshfeghi, Phoenix Stout, Jonghwee Park, Dinhan Pham, and Shuxin Tan for their assistance with device testing and system design. R.S. acknowledges support from the UCLA-Caltech Medical Scientist Training Program (NIGMS T32 GM008042).

■ REFERENCES

(1) Byrne, R.; Diamond, D. Chemo/bio-sensor networks. *Nat. Mater.* **2006**, *5*, 421−424.

(2) Lin, H.; Zhao, Y.; Lin, S.; Wang, B.; Yeung, C.; Cheng, X.; Wang, Z.; Cai, T.; Yu, W.; King, K.; Tan, J.; Salahi, K.; Hojaiji, H.; Emaminejad, S. A rapid and low-cost fabrication and integration scheme to render 3D microfluidic architectures for wearable biofluid sampling, manipulation, and sensing. *Lab Chip* **2019**, *19*, 2844−2853.

(3) Lin, H.; Hojaiji, H.; Lin, S.; Yeung, C.; Zhao, Y.; Wang, B.; Malige, M.; Wang, Y.; King, K.; Yu, W.; Tan, J.; Wang, Z.; Cheng, X.; Emaminejad, S. A wearable electrofluidic actuation system. *Lab Chip* **2019**, *19*, 2966−2972.

(4) Heikenfeld, J.; Jajack, A.; Rogers, J.; Gutruf, P.; Tian, L.; Pan, T.; Li, R.; Khine, M.; Kim, J.; Wang, J.; et al. Wearable sensors: modalities, challenges, and prospects. *Lab Chip* **2018**, *18*, 217−248.

(5) Lee, H.; Choi, T. K.; Lee, Y. B.; Cho, H. R.; Ghaffari, R.; Wang, L.; Choi, H. J.; Chung, T. D.; Lu, N. S.; Hyeon, T.; Choi, S. H.; Kim, D. H. A graphene-based electrochemical device with thermoresponsive microneedles for diabetes monitoring and therapy. *Nat. Nanotechnol.* **2016**, *11*, 566−572.

(6) Koh, A.; Kang, D.; Xue, Y.; Lee, S.; Pielak, R. M.; Kim, J.; Hwang, T.; Min, S.; Banks, A.; Bastien, P.; Manco, M. C.; Wang, L.; Ammann, K. R.; Jang, K. I.; Won, P.; Han, S.; Ghaffari, R.; Paik, U.; Slepian, M. J.; Balooch, G.; Huang, Y. G.; Rogers, J. A. A soft, wearable microfluidic device for the capture, storage, and colorimetric sensing of sweat. *Sci. Transl. Med.* **2016**, *8*, No. 366ra165.

(7) Bariya, M.; Nyein, H. Y. Y.; Javey, A. Wearable sweat sensors. *Nat. Electron.* **2018**, *1*, 160−171.

(8) Heikenfeld, J. Non-invasive Analyte Access and Sensing through Eccrine Sweat: Challenges and Outlook circa 2016. *Electroanalysis* **2016**, *28*, 1242−1249.

(9) Martín, A.; Kim, J.; Kurniawan, J. F.; Sempionatto, J. R.; Moreto, J. R.; Tang, G. D.; Campbell, A. S.; Shin, A.; Lee, M. Y.; Liu, X. F.; Wang, J. Epidermal Microfluidic Electrochemical Detection System: Enhanced Sweat Sampling and Metabolite Detection. *ACS Sens.* **2017**, *2*, 1860−1868.

(10) Gao, W.; Emaminejad, S.; Nyein, H. Y. Y.; Challa, S.; Chen, K. V.; Peck, A.; Fahad, H. M.; Ota, H.; Shiraki, H.; Kiriya, D.; Lien, D. H.; Brooks, G. A.; Davis, R. W.; Javey, A. Fully integrated wearable sensor arrays for multiplexed in situ perspiration analysis. *Nature* **2016**, *529*, 509−514.

(11) Zhang, Y.; Guo, H. X.; Kim, S. B.; Wu, Y. X.; Ostojich, D.; Park, S. H.; Wang, X. J.; Weng, Z. Y.; Li, R.; Bandodkar, A. J.; Sekine, Y.; Choi, J.; Xu, S.; Quaggin, S.; Ghaffari, R.; Rogers, J. A. Passive sweat collection and colorimetric analysis of biomarkers relevant to kidney disorders using a soft microfluidic system. *Lab Chip* **2019**, *19*, 1545−1555.

(12) Simmers, P.; Li, S. K.; Kasting, G.; Heikenfeld, J. Prolonged and localized sweat stimulation by iontophoretic delivery of the slowly-metabolized cholinergic agent carbachol. *J. Dermatol. Sci.* **2018**, *89*, 40−51.

(13) Emaminejad, S.; Gao, W.; Wu, E.; Davies, Z. A.; Nyein, H. Y. Y.; Challa, S.; Ryan, S. P.; Fahad, H. M.; Chen, K.; Shahpar, Z.; Talebi, S.; Milla, C.; Javey, A.; Davis, R. W. Autonomous sweat extraction and analysis applied to cystic fibrosis and glucose

monitoring using a fully integrated wearable platform. *Proc. Natl. Acad. Sci. U.S.A.* **2017**, *114*, 4625−4630.

(14) Kim, J.; Campbell, A. S.; de Avila, B. E. F.; Wang, J. Wearable biosensors for healthcare monitoring. *Nat. Biotechnol.* **2019**, *37*, 389−406.

(15) Jajack, A.; Brothers, M.; Kasting, G.; Heikenfeld, J. Enhancing glucose flux into sweat by increasing paracellular permeability of the sweat gland. *PLoS One* **2018**, *13*, No. e0200009.

(16) Sonner, Z.; Wilder, E.; Heikenfeld, J.; Kasting, G.; Beyette, F.; Swaile, D.; Sherman, F.; Joyce, J.; Hagen, J.; Kelley-Loughnane, N.; Naik, R. The microfluidics of the eccrine sweat gland, including biomarker partitioning, transport, and biosensing implications. *Biomicrofluidics* **2015**, *9*, No. 031301.

(17) Leggett, R.; Lee-Smith, E. E.; Jickells, S. M.; Russell, D. A. "Intelligent" fingerprinting: Simultaneous identification of drug metabolites and individuals by using antibody-functionalized nanoparticles. *Angew. Chem., Int. Ed.* **2007**, *46*, 4100−4103.

(18) Hazarika, P.; Russell, D. A. Advances in Fingerprint Analysis. *Angew. Chem., Int. Ed.* **2012**, *51*, 3524−3531.

(19) Costa, C.; Webb, R.; Palitsin, V.; Ismail, M.; de Puit, M.; Atkinson, S.; Bailey, M. J. Rapid, Secure Drug Testing Using Fingerprint Development and Paper Spray Mass Spectrometry. *Clin. Chem.* **2017**, *63*, 1745−1752.

(20) Burns, M.; Baselt, R. C. Monitoring drug use with a sweat patch: an experiment with cocaine. *J. Anal. Toxicol.* **1995**, *19*, 41−48.

(21) Dolan, K.; Rouen, D.; Kimber, J. An overview of the use of urine, hair, sweat and saliva to detect drug use. *Drug Alcohol Rev.* **2004**, *23*, 213−217.

(22) Dutkiewicz, E. P.; Lin, J. D.; Tseng, T. W.; Wang, Y. S.; Urban, P. L. Hydrogel Micropatches for Sampling and Profiling Skin Metabolites. *Anal. Chem.* **2014**, *86*, 2337−2344.

(23) Dutkiewicz, E. P.; Chiu, H. Y.; Urban, P. L. Micropatch-arrayed pads for non-invasive spatial and temporal profiling of topical drugs on skin surface. *J. Mass Spectrom.* **2015**, *50*, 1321−1325.

(24) Kuwayama, K.; Tsujikawa, K.; Miyaguchi, H.; Kanamori, T.; Iwata, Y. T.; Inoue, H. Time-course measurements of caffeine and its metabolites extracted from fingertips after coffee intake: a preliminary study for the detection of drugs from fingerprints. *Anal. Bioanal. Chem.* **2013**, *405*, 3945−3952.

(25) Nagamine, K.; Mano, T.; Nomura, A.; Ichimura, Y.; Izawa, R.; Furusawa, H.; Matsui, H.; Kumaki, D.; Tokito, S. Noninvasive Sweat-Lactate Biosensor Emplsoying a Hydrogel-Based Touch Pad. *Sci. Rep.* **2019**, *9*, No. 10102.

(26) Schmidt, B.; Roberts, R. S.; Davis, P.; Doyle, L. W.; Barrington, K. J.; Ohlsson, A.; Solimano, A.; Tin, W. Caffeine Apnea Prematurity Trial, G. Caffeine therapy for apnea of prematurity. *N. Engl. J. Med.* **2006**, *354*, 2112−2121.

(27) Dobson, N. R.; Liu, X. X.; Rhein, L. M.; Darnall, R. A.; Corwin, M. J.; McEntire, B. L.; Ward, R. M.; James, L. P.; Sherwin, C. M. T.; Heeren, T. C.; Hunt, C. E. Salivary caffeine concentrations are comparable to plasma concentrations in preterm infants receiving extended caffeine therapy. *Br. J. Clin. Pharmacol.* **2016**, *82*, 754−761.

(28) Derbyshire, P. J.; Barr, H.; Davis, F.; Higson, S. P. J. Lactate in human sweat: a critical review of research to the present day. *J. Physiol. Sci.* **2012**, *62*, 429−440.

(29) Jia, W.; Bandodkar, A. J.; Valdes-Ramirez, G.; Windmiller, J. R.; Yang, Z. J.; Ramirez, J.; Chan, G.; Wang, J. Electrochemical Tattoo Biosensors for Real-Time Noninvasive Lactate Monitoring in Human Perspiration. *Anal. Chem.* **2013**, *85*, 6553−6560.

(30) Khodagholy, D.; Curto, V. F.; Fraser, K. J.; Gurfinkel, M.; Byrne, R.; Diamond, D.; Malliaras, G. G.; Benito-Lopez, F.; Owens, R. M. Organic electrochemical transistor incorporating an ionogel as a solid state electrolyte for lactate sensing. *J. Mater. Chem.* **2012**, *22*, 4440−4443.

(31) Tai, L. C.; Gao, W.; Chao, M. H.; Bariya, M.; Ngo, Q. P.; Shahpar, Z.; Nyein, H. Y. Y.; Park, H.; Sun, J.; Jung, Y.; Wu, E.; Fahad, H. M.; Lien, D. H.; Ota, H.; Cho, G.; Javey, A. Methylxanthine Drug Monitoring with Wearable Sweat Sensors. *Adv. Mater.* **2018**, *30*, No. 1707442.

(32) Ismail, M.; Stevenson, D.; Costa, C.; Webb, R.; de Puit, M.; Bailey, M. Noninvasive Detection of Cocaine and Heroin Use with Single Fingerprints: Determination of an Environmental Cutoff. *Clin. Chem.* **2018**, *64*, 909−917.

(33) Ohshima, Y.; Shimizu, H.; Yanagishita, T.; Watanabe, D.; Tamada, Y.; Sugenoya, J.; Tsuda, T.; Matsumoto, Y. Changes in Na+, K+ concentrations in perspiration and perspiration volume with alternating current iontophoresis in palmoplantar hyperhidrosis patients. *Arch. Dermatol. Res.* **2008**, *300*, 595−600.

(34) Kim, J.; Farahmand, M.; Dunn, C.; Davies, Z.; Frisbee, E.; Milla, C.; Wine, J. J. Evaporimeter and bubble-imaging measures of sweat gland secretion rates. *PLoS One* **2016**, *11*, No. e0165254.

(35) Heikenfeld, J.; Jajack, A.; Feldman, B.; Granger, S. W.; Gaitonde, S.; Begtrup, G.; Katchman, B. A. Accessing analytes in biofluids for peripheral biochemical monitoring. *Nat. Biotechnol.* **2019**, *37*, 407−419.

(36) Ptolemy, A. S.; Tzioumis, E.; Thomke, A.; Rifai, S.; Kellogg, M. Quantification of theobromine and caffeine in saliva, plasma and urine via liquid chromatography-tandem mass spectrometry: A single analytical protocol applicable to cocoa intervention studies. *J. Chromatogr. B* **2010**, *878*, 409−416.

(37) Zhang, Y.; Mehrotra, N.; Budha, N. R.; Christensen, M. L.; Meibohm, B. A tandem mass spectrometry assay for the simultaneous determination of acetaminophen, caffeine, phenytoin, ranitidine, and theophylline in small volume pediatric plasma specimens. *Clin. Chim. Acta* **2008**, *398*, 105−112.

(38) Taylor, N. A.; Machado-Moreira, C. A. Regional variations in transepidermal water loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans. *Extreme Physiol. Med.* **2013**, *2*, No. 4.

(39) Fruhstorfer, H.; Abel, U.; Garthe, C. D.; Knuttel, A. Thickness of the stratum corneum of the volar fingertips. *Clin. Anat.* **2000**, *13*, 429−433.

(40) Wen, X.; Wang, B.; Huang, S.; Liu, T.; Lee, M. S.; Chung, P. S.; Chow, Y. T.; Huang, L. W.; Monbouquette, H. G.; Maidment, N. T.; Chiou, P. Y. Flexible, multifunctional neural probe with liquid metal enabled, ultra-large tunable stiffness for deep-brain chemical sensing and agent delivery. *Biosens. Bioelectron.* **2019**, *131*, 37−45.

(41) Wang, B.; Koo, B.; Huang, L. W.; Monbouquette, H. G. Microbiosensor fabrication by polydimethylsiloxane stamping for combined sensing of glucose and choline. *Analyst* **2018**, *143*, 5008−5013.

(42) Jea, T. Y.; Chavan, V. S.; Govindaraju, V.; Schneider, J. K. Security and Matching of Partial Fingerprint Recognition Systems, In *Biometric Technology for Human Identification*, Proceedings of SPIE, 2004; pp 39−50.

DOI: 10.1021/acssensors.9b01727
ACS Sens. XXXX, XXX, XXX−XXX