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Nicotine Monitoring with Wearable Sweat Band

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ABSTRACT: The tobacco epidemic is a public health threat that has taken a heavy toll of lives around the globe each year. Smoking affects both the smokers and those who are exposed to secondhand smoke, and careful tracking of exposure can be key to mitigating the potential hazards. For smokers, the variation of chemical compositions between commercial cigarettes has led to ambiguity in estimating the health risks, both for active smokers and others involuntarily exposed to tobacco smoke and byproducts. In this regard, sweat possesses an attractive opportunity to monitor smoke exposure due to sweat's abundance in biomolecules and its great accessibility. Here we present a wearable sweat band to monitor nicotine, a prominent ingredient in cigarettes, as a viable way to quantitatively assess a wearer's exposure to smoking. Both smokers and normal subjects are tested to demonstrate the use of this device for smoke-related health monitoring. Our results exhibit confirmable, elevated nicotine levels in sweat for subjects inhaling cigarette smoke. This continuous and personalized sweat sensing device is leverage to monitor smoke pollution for a potentially broad population.

KEYWORDS: *nicotine monitoring, electrochemical devices, wearable sweat sensors, flexible electronics, nano-dendrites, self-assembled monolayer*

Tobacco exposure is a leading modern-day epidemic that has claimed more than eight million lives worldwide every year from direct tobacco use and indirect exposure to secondhand smoke.¹ These statistics convey the broad scope of people affected by smoking and the need to spearhead effective measures against

smoke pollution. The adverse physiological and psychological side effects of ingredients in cigarettes can lead to sleep disorders, cardiovascular diseases, addictions, diabetes, and respiratory dysfunctions.¹⁻⁶ Therefore, tobacco smoke that permeates into various environments presents a serious public health threat that merits careful attention. The cumulative exposure to secondhand smoke in public spaces and private environments can be hard to quantify, which leads to the underestimation of irreversible health consequences on nonsmoking individuals.⁷ Therefore, a tobacco monitoring device is necessary to properly evaluate the scope of health risks among nonsmoking populations. The conventional technique to detect tobacco smoke mounts a sensor at a fixed location, which lacks the flexibility to determine smoke exposure on specific individuals and offer personalized alerts.⁸ Commercially available tobacco test strips allow users to analyze urine and saliva specimens to monitor nicotine exposure at separate time points.⁹ However, a test strip's inability for continuous monitoring and inconvenient processing steps make it practically challenging to capture a complete picture of an individual's exposure to tobacco, and thus making efforts of tailoring preventive strategies difficult. To address the abovementioned obstacles, we present a wearable sweat band (s-band) for nicotine monitoring to bridge the technological gap between mounted tobacco detectors and discrete nicotine testing strips. In this work, sweat is distinctively chosen as the sensing bio-fluid because of its abundance in biomolecules and its great accessibility.¹⁰⁻¹⁷ Nicotine is selected as an attractive target due to its presence in nearly all tobacco products.²⁰ Like many xenobiotic biomolecules, nicotine has been demonstrated to excrete in sweat, with its concentrations positively correlated with smoking.^{17-19,21} Additionally, under normal smoking conditions, sweat nicotine concentration is expected to reach up to several micro-molars, which is within the detectable range with existing nicotine sensing technologies.^{17,21-25} This intimate connection between individuals' exposure to nicotine and their sweat nicotine levels provides us the framework to design a wearable s-band for nicotine monitoring. An electrochemical technique is chosen for sensor development because of its advantages for high sensitivity, good selectivity, compatibility with electronics, and low costs.¹⁰⁻¹⁶ Our approach addresses the shortcomings of the current smoke monitoring devices and paves a way to continuous, real-time, and on-site

secondhand smoke monitoring. Figure 1a shows the nicotine s-band consisted of a flexible electrode array connected to a compact printed circuit board. During an on-body use, the s-band is worn on the subject's forearm to detect the level of nicotine in human sweat after nicotine inhalation. The cross sectional layers of the electrode array are shown in Figure 1b. On a polyethylene terephthalate (PET) film, a gold working electrode is grown with gold nano-dendrites and modified with cytochrome P450 2B6 (CYP2B6), a nicotine oxidizing enzyme.²⁶⁻²⁹ A silver/silver chloride reference electrode and a gold counter electrode are also integrated. Figure 1c shows a zoom-in view of the functionalized working electrode. Gold nano-dendrites are directly grown on the gold electrode, and a self-assembled monolayer of 11-mercaptopundecanoic acid (MUA) is coated on top.^{30,31} The MUA then immobilizes the enzyme by covalently bonding with CYP2B6.³²⁻³⁴ The successful addition of each layer is supported by the electrochemical impedance spectroscopy, shown in Figure S1.

The gold nano-dendritic structure of the functionalized working electrode was captured with a scanning electron microscope (SEM), shown in Figure 2a. The gold nano-dendrites increase the surface area of the electrode to improve the sensitivity and stability of the sensor.^{13,30} Figure 2b shows the oxidation peak from cyclic voltammetry (CV) of the functionalized electrode in phosphate-buffered saline (PBS) solution, with the subsequent addition of nicotine (0 – 30 μM). The oxidation peak increases in its height with increasing concentration of nicotine and is centered at 0.8 V, the potential chosen for amperometric testing.²²⁻²⁵ For the purpose of comparison, Figure S2 shows nicotine's distinct peak relative to other xenobiotic molecules such as caffeine and levodopa. Figure 2c shows a representative plot of the amperometric response of the functionalized electrode to nicotine, and its corresponding current-to-concentration calibration curve is shown in Figure 2d. The sensitivity is calculated to be 4.3 nA/ μM , which is on par with the state-of-the-art nicotine sensors.^{22,23} Figure 2e shows a histogram plot of the sensor-to-sensor variation in terms of their sensitivities. The standard deviation is found to be 1.4 nA/ μM , which contributes up to 30 % of deviation that dominates the linear calibration curve's uncertainty. For a high sweat nicotine level of 5 μM , this translates to about 1.6 μM of uncertainty. The effects of pH and temperature can potentially influence the performance of enzymatic-based sensors.¹¹ Figure 2f

shows the functionalized electrode's response to varying degrees of pH, prepared using McIlvaine buffer. The range of pH (5 – 7) is selected in the physiologically relevant range of human sweat, and the corresponding sensitivities have a standard deviation of 0.9 nA/ μ M, which is within the standard deviation calculated from sensor-to-sensor variations.¹¹ The effect of the temperature is demonstrated in Figure 2g, where the functionalized electrode is first spiked with 5 μ M of nicotine, followed by gradually increasing the temperature from 20 °C to 40 °C. The result shows the sensitivity to temperature is 2.2 nA/°C, which is used to calibrate the amperometric readings prior to conversion into nicotine concentrations. The functionalized electrode's responses to interferences are tested in Figure 2h, with the addition of common biomolecules such as glucose (100 μ M), ascorbic acid (10 μ M), uric acid (20 μ M), and caffeine (10 μ M). The interferences are found to contribute to about $\pm$ 1 μ M of uncertainty when compared to the sensor's response to nicotine.

To characterize the sensor performance in human sweat, Figure 3 shows the functionalized electrode's response in sweat solutions. Figure 3a shows a schematic of the sensing of nicotine molecules in sweat solutions, where the CYP2B6 enzyme oxidizes nicotine into nicotine iminium ion.²⁶⁻²⁹ Figure 3b shows the amperometric response of nicotine being oxidized at a constant applied potential of 0.8 V. By adding nicotine into the sweat solution, the Faradaic current increases gradually, and the current-to-concentration calibration is shown in Figure 3c. The sensitivity is found to be 4.4 nA/ μ M, which is similar to the sensitivity tested in PBS.

Figure 4 explores the possibility of utilizing the nicotine s-band to detect an individual's exposure to nicotine. Both nonsmoking subjects and smokers volunteered for this study. The subjects were permitted to generate sweat via various approaches, such as cycling, intense walking, and sports competition. Nonsmokers had no tobacco exposure, and their sweat solutions were directly analyzed after perspiration. Regular smokers started exercising within five minutes after they each smoked a standard cigarette. Their sweat solutions were similarly analyzed when they started sweating, typically five to ten minutes after the start of their exercise. Figure 4a shows the experimental design using the s-band for nicotine monitoring. The subjects smoked before engaging in physical activities to

generate sweat. Sweat nicotine levels were then analyzed, as described in the Experimental Section. In Figure 4b, different exercise trials, including both nonsmokers and smokers, were performed to compare the sweat nicotine levels between controls and subjects exposed to nicotine. Our results show consistently low concentrations for nonsmokers, with average nicotine levels below the limit of detection. The smokers' sweat samples exhibit elevated nicotine concentrations with an average value of 4.8 μM , which is within the concentration range previously observed.^{17,21} One smoker shows a low nicotine level. This is potentially due to various factors, such as metabolic rates, the amount of nicotine content in cigarettes, and the degree of nicotine inhalation during smoking. Nevertheless, on average, we observe a good distinction between the sweat nicotine concentration between the nonsmokers and smokers. This set of experiments demonstrates the potential for distinguishing the subjects' exposure to nicotine using sweat solutions. Prior to on-body trials, the nicotine sensor is tested for a long duration, as shown in Figure S3. The signal-to-noise ratio (SNR) is calculated by dividing the change in amperometric current with the addition of 5 μM nicotine (about half of the maximum observed value of sweat nicotine concentration after regular smoking) by the amplitude of current fluctuation.¹⁷ Based on Figure S3, the SNR is calculated to be 4, which corresponds to about 1.3 μM of uncertainty due to noise. The sensor's overall drift is small, corresponding to less than 0.16 $\mu\text{M}/\text{min}$. The detection limit is then calculated by looking at the uncertainty calculated earlier due to the interference (1 μM), sensor-to-sensor variation and linear calibration (1.6 μM), noise level (1.3 μM), and sensor drift over the average duration of on-body testing (1.3 μM) and assigning the largest value. The precision of our measurement is then constrained by the detection limit of 1.6 μM .

In order to demonstrate the use of nicotine s-band for continuous nicotine monitoring, multiple subjects were recruited for sweat analysis. Figure 5a shows the experimental design with the subjects going through cycling on an ergometer. The on-body s-band seamlessly measures the nicotine level over time, with time zero indicating the start of sweating. Figure 5b shows the images of a subject cycling on an ergometer and a zoom-in view of the s-band. Two types of on-body experiments were performed. The first group includes healthy nonsmokers, and they were instructed to cycle on the ergometer for half an hour or until fatigue. As

shown in Figure 5c, the nonsmokers' sweat profiles demonstrate a low nicotine concentration typically below the detection limit. Figure 5d monitors the smokers' sweat nicotine profiles. The smokers smoke one cigarette, which contains about 13 mg of nicotine, right before cycling on an ergometer.³⁵⁻³⁷ Their sweat profiles are then analyzed to evaluate the concentrations of nicotine. The nicotine concentrations indicate an average peak level of 5.8 μM . In both smokers' profiles, the sweat nicotine concentrations increase in the beginning, which may be attributed to the absorption of nicotine that typically happens on the order of minutes. After reaching their respective peaks, we do not observe obvious variation in their nicotine concentrations. This may be attributed to nicotine's slow metabolic decay, with the expected half-life on the order of hours.³⁸⁻⁴⁰ Comparing Figure 5c and 5d, the distinctions between sweat profiles from smokers and nonsmokers show that the *s*-band can pick up the inhalation of nicotine.

In conclusion, we have demonstrated a noninvasive and wearable *s*-band capable of monitoring nicotine. Our work combines several layers of modification on the working electrode to allow for effective sensing. By integrating the physical properties of gold nano-dendrites and the chemical bonding enabled through the self-assembled monolayers, the sensor exhibits good sensitivity and stability that are essential for detecting nicotine in sweat solutions. This amenable platform can serve as a template for future sensor designs. We have also investigated the sweat nicotine concentrations between nonsmokers and smokers, as well as their nicotine profiles over time. Our observation shows that the nicotine *s*-band is capable of directly picking up nicotine levels in real-time for those inhaling cigarette smoke.

Importantly, the *s*-band overcomes the shortcomings of other commercial tobacco detectors that are limited by their lack of mobility and inability for continuous measurements. From the society's point of view, the *s*-band provides the general population an excellent device to monitor secondhand smoke and allows for effective policymaking related to smoke prevention. In tandem with existing sensor networks and big data analysis, the nicotine *s*-band can remarkably impact the healthcare industry by enriching the bioinformatics available to its users.

We envision that the nicotine *s*-band's long-term monitoring capability and its application geared toward the general public will significantly expand the realm of wearable sensing technologies and chart new territories for biomedical discoveries. Further, the longitudinal and cross sectional studies enabled through the nicotine *s*-band will provide us with profound insight into various chronic and underlying pathological conditions that can be explored for preventive care and personalized medication.

EXPERIMENTAL SECTION

Functionalization of electrodes to fabricate the nicotine sensor. Each sensor consists of a working electrode, a reference electrode, and a counter electrode, all prepared on a PET film. The sensors were initially patterned by photolithography technique with positive photoresist (Shipley Microposit S1818), and chromium (30 nm) and gold (50 nm) were evaporated subsequently with electron-beam to form a conductive three-electrode system. The working electrode was modified by growing gold nano-dendrites by immersing the electrode in a mixture solution of 50 mM chloroauric acid and 50 mM hydrochloric acid and applying an alternating potential via a Gamry Electrochemical Potentiostat (signal type: square wave; amplitude: 1 V; DC offset -1 V; signal frequency: 50 Hz; cycles: 1500). The electrodes were gently immersed in deionized water for ten seconds and left in room temperature for two hours. Afterward, the electrodes were immersed in 11-mercaptoundecanoic acid (MUA) solution (0.5 g MUA, 1 L ethanol) for 16 hours to allow self-assembled monolayers to form on top of the gold surface. Then, the electrodes were incubated in room temperature in a mixture of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) solution (283 μ L EDC, 62 mg NHS, 4 mL PBS) for 15 minutes. Afterward, the electrodes were incubated in a CYP2B6 enzyme solution for 1 hour before drop-casting the same solution on top of the working electrode (2.5 μ L). The solution was prepared by mixing CYP2B6 (Sigma-Aldrich. 37.8 mg) in PBS (1 mL). The image of CYP2B6 in Figure 1 was created with www.rcsb.org.⁴¹ The reference electrode was prepared by pasting silver/silver chloride ink onto the existing, evaporated gold electrode to provide a stable reference. The counter electrode was not modified further and remained as a simple gold electrode. The electrodes

were left in room temperature for overnight for the enzyme and silver/silver chloride paste to stabilize prior to testing with a potentiostat.

Nicotine sensor's characterization and calibration in buffer solution. To characterize the functionalized working electrode, a commercial silver/silver chloride reference electrode and platinum wire were used to form a complete three-electrode electrochemical system in a PBS solution. CHI 1230C potentiostat (CH Instrument) was used for electrochemical measurements. Different concentrations of nicotine were subsequently added into the solution to obtain the corresponding CV and amperometric response. The sensor's response to temperature variation was performed by heating up the nicotine solution with a hotplate and measuring the solution's temperature with a thermometer. The amperometric response was captured once the solution reached the desired temperature. The sensor's response to pH was tested in McIlvaine buffer, which had an easily tunable range of pH. The slight difference in baseline current was normalized between McIlvaine buffer with different pH. The interference test was performed by the subsequent addition of selected categories of common biomolecules in human bio-fluid. The concentration of each interferent was determined to be within the normal physiological range.

Nicotine sensor's characterization in sweat. The characterization of the on-body nicotine sensor in sweat solutions, shown in Figure 3, was conducted by first collecting sweat samples from volunteers. The amperometric response of the sensor was performed by directly connecting the functionalized working, reference, and counter electrodes to CHI 1230C potentiostat. The average value at each nicotine concentration in Figure 3b was used for plotting Figure 3c.

Sweat collection and nicotine concentration analysis. The sweat samples were collected via various methods. Volunteers were recruited from around the City of Berkeley. Sweat was generated by intense walking, cycling (Kettler E3 Upright Exercise Bike), or playing basketball. After perspiration began, a 200- μ L centrifuge tube was used to collect sweat samples from the volunteers' foreheads. The volunteers cleaned their foreheads with gauze prior to each subsequent collection with a new centrifuge tube. Prior to testing the collected sweat samples, the nicotine sensor was characterized following the procedure described in the nicotine sensor's characterization section. Afterward, the collected sweat samples

were evaluated with amperometry to convert the amperometric responses into nicotine concentrations. The institutional review board number approved at the University of California, Berkeley, is CPHS 2014-08-6636.

In situ sweat nicotine analysis. The volunteers engaged in cycling on a stationary ergometer at a biking (Kettler E3 Upright Exercise Bike) power of 100 W. The nicotine sensors, packaged in terms of the s-band, were mounted on top of the subjects' wrists over the entire duration of the cycling. The temperature calibration curve was used to convert the amperometric responses of the sensors operated at the skin temperatures of the subjects back to the current levels at room temperature, at which the sensors were characterized. The current levels were then converted to nicotine concentration using the calibration curves obtained prior to on-body exercise experiments. The raw data converted into nicotine concentration was further filtered (MATLAB Hampel Function and Smooth Function) on a personal computer.

Cigarette smoking. The smoking subjects smoked a cigarette right before engaging in physical exercise. A standard cigarette consists of approximately 13 mg of nicotine.³⁵⁻³⁷

Statistical procedures. During sensor characterization, statistical procedures were used. The sensors' sensitivities to nicotine are incorporated into a histogram plot in Figure 2e (10 sensors). The mean, median, and standard deviation are 4.4 nA/ μ M, 4.6 nA/ μ M, and 1.4 nA/ μ M, respectively. A representative sensor was chosen for figure 2d, with a sensitivity of 4.3 nA/ μ M. In subsequent sensor characterization in Figure 3 with sweat solution, the sensor is retested for confirmation purposes if its sensitivity shows a value that is more than a standard deviation away from the statistical mean.

ASSOCIATED CONTENT

Supporting Information Available: The following files are available free of charge. Supporting Information for Publication.

Electrochemical impedance spectroscopy of the gold electrode under various stages of modification; Oxidation peaks for selected xenobiotic molecules measured with cyclic voltammetry; Long-term stability test of a functionalized working electrode

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Author Contributions

L.-C.T. and A.J. designed the experiments. L.-C.T., C.H.A., and A.J. contributed to data collection, analysis and interpretation. L.-C.T., C.H.A., H.Y.Y.N., W.J., M.B., Y.L., and L.L. contributed to sensor fabrication and preparation. L.-C.T. and A. J. wrote the paper and all authors provided feedback.

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Figure 1. The design and application of the *s*-band. (a) Optical image of a nicotine *s*-band and a schematic showing the application of the wearable device on monitoring secondhand smoke. (b) A cross-sectional illustration of the flexible sensor patch, which consists of a functionalized working electrode modified with CYP2B6 enzyme, a reference electrode pasted with silver/silver chloride, and a counter electrode. (c) Zoom-in schematics of the functionalized working electrode, which has gold nano-dendrites grown on top of the gold substrate. The nano-dendrites are connected to CYP2B6, a nicotine oxidizing enzyme, via a self-assembled monolayer of 11-mercaptoundecanoic acid (MUA).

Figure 2. Characterization of the functionalized working electrode in PBS. (a) SEM image of the gold nano-dendrites. (b) Zoom-in view of a CV of nicotine (NIC) dissolved in PBS. (c) The amperometric response of nicotine dissolved in PBS and (d) linear relationship of the amperometric current and nicotine concentration. (e) Sensor-to-sensor variations in terms of sensitivities. (f) The effect of pH on sensor sensitivities. (g) The effect of temperature on the amperometric response. (h) The effect of potential interferences, such as glucose (G), ascorbic acid (AA), uric acid (UA), and caffeine (CAF), on the amperometric response.

Figure 3. Characterization of the nicotine sensor in sweat solutions. (a) Sensing nicotine molecules in sweat solutions. Sweat nicotine is oxidized via CYP2B6 on the working electrode (WE) into nicotine iminium ion. The reference electrode (RE) and the counter electrode (CE) are also shown.

This oxidation generates a current that is measured in terms of (b) the amperometric response to nicotine. (c) The corresponding calibration curve.

Figure 4. Nicotine monitoring via exercise-induced sweat. Sweat samples are collected after smoking (for smoking subjects) and subsequent exercise. (a) Schematics of the experiment. (b) Nicotine concentrations measured for nonsmokers and smokers.

Figure 5. On-body nicotine monitoring via exercise-induced sweat. (a) Schematics of the experiment and (b) optical images of the experiment. Examples of sweat nicotine concentrations for subjects cycling on an ergometer (c) without smoking, and (d) with smoking.

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