Wearable microsensor array for multiplexed heavy metal monitoring of body fluids

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ABSTRACT: A flexible and wearable microsensor array is described for simultaneous multiplexed monitoring of heavy metals in human body fluids. Zn, Cd, Pb, Cu, and Hg ions are chosen as target analytes for detection via electrochemical square wave anodic stripping voltammetry (SWASV) on Au and Bi microelectrodes. The oxidation peaks of these metals are calibrated and compensated by incorporating a skin temperature sensor. High selectivity, repeatability, and flexibility of the sensor arrays are presented. Human sweat and urine samples are collected for heavy metal analysis, and measured results from the microsensors are validated through inductively coupled plasma mass spectrometry (ICP-MS). Real-time on-body evaluation of heavy metal (e.g., zinc and copper) levels in sweat of human subjects by cycling is performed to examine the change in concentrations with time. This platform is anticipated to provide insightful information about an individual’s health state such as heavy metal exposure and aid the related clinical investigations.

KEYWORDS: flexible electronics, wearable biosensors, heavy metals monitoring, sweat, multiplexed sensing, temperature compensation
Russell syndrome, Minamata disease, and acrodynia, to name a few.\textsuperscript{26,27} Therefore, determining one’s exposure to such heavy metals can offer important insights into a person’s health. Human sweat and urine are known to be the most important sources for detoxification of heavy metals;\textsuperscript{26} therefore, examination of sweat and urine heavy metals can assist toxicological and therapeutic studies.

Detection of heavy metals in body fluids is challenging due to their extremely low concentrations (on the order of $\mu g/L$). Conventional heavy metals analysis procedures including atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS) rely on bulky and expensive analytical instruments.\textsuperscript{30,31} The effective preconcentration/deposition step and advanced electrochemical measurements of the accumulated analytes make anodic stripping analysis the most sensitive and effective electroanalytical technique.\textsuperscript{32} The stripping-voltammetric measurements of trace metals in sweat have been reported using collected

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**Figure 1.** Flexible microsensor arrays for multiplexed heavy metals analysis. (a) Schematic showing the concept of deposition and stripping on microelectrodes. (b) Schematic showing the composition of the microsensor array. (c) Optical image of a flexible sensor array interfacing with a flexible printed circuit connector.

**Figure 2.** Fabrication process of flexible microsensor arrays. (a) Cleaning of PET substrate using acetone, isopropanol, and O$_2$ plasma etching. (b) Patterning of Cr/Au electrodes via photolithography, e-beam evaporation, and lift-off in acetone. (c) Parylene insulating layer deposition, photolithography, and O$_2$ plasma etching of parylene in the electrode areas. (d) E-beam deposition of Ag layer followed by lift-off in acetone. (e) Photolithography and e-beam deposition of Bi layer followed by lift-off in acetone. (f) Nafion coating and PDMS bonding. (g) An optical image of a flexible electrode array. (h) Microscopic image of a multiplexed microsensor array.
sweat samples. Such use of collected sweat may be subject to inaccuracy due to sample contamination and sweat evaporation. In addition, it cannot yield real-time information on dynamic events. Recently, on-body detection of zinc in sweat has also been reported using printed epidermal tattoos. Given the importance or toxicity of a variety of heavy metals to human skin, it is very attractive to perform simultaneous multiplexed screening of heavy metals in sweat and do proper signal calibrations to ensure accurate measurements.

In this paper, we demonstrate a flexible multiplexed trace metal monitoring device to extract useful information on heavy metal levels in body fluids such as sweat and urine. It can also be used as a wearable device for real-time monitoring of the heavy metals in human sweat. A microsensor array is utilized to simultaneously and selectively measure multiple heavy metals including Zn, Cd, Pb, Cu, and Hg through square wave anodic stripping voltammetry (SWASV), as well as the skin temperature to calibrate heavy metal sensors’ readings in real-time (Figure 1a). The microsensor array consists of four microelectrodes: biocompatible gold and bismuth working electrodes (WE), a silver reference electrode (RE), and a gold counter/auxiliary electrode (CE) (Figure 1b). In addition, a resistance-based skin temperature sensor based on evaporated Cr/Au microlines is integrated into the system to compensate the active macromolecules such as proteins. A polydimethylsiloxane (PDMS) layer of parylene C is chosen as an insulation layer to ensure reliable measurement by preventing electrical contact of the conducting metal lines with body fluids and skin. Photo- lithography and O2 plasma etching are used to define the electrode area (100 μm × 1200 μm). After patterning the Ag reference, Bi and Au working electrodes, a thin Nafion coating is used as an antifouling layer to minimize biofouling of surface-active macromolecules such as proteins. A polydimethylsiloxane (PDMS) well (6 mm diameter and 1 mm thickness) was also bonded on top of the sensor array by soft lithography and O2 plasma etching. This ensures sweat accumulation (20–30 μL) such that a stable and reliable stripping analysis can be done. The microelectrodes array is then connected to a

The choice of the working electrode is crucial for successful stripping analysis. The ideal material for working electrode should offer an effective preconcentration, a favorable redox reaction of the target metal, reproducible and renewable surface, and a low background current over a wide potential range. Although mercury has been the most explored electrode for many stripping applications, it is not desired for wearable biosensors given its toxicity and volatility. In contrast, the attractive stripping voltammetric performance and biocompatibility of the bismuth and gold electrodes can serve as the ideal candidate for developing wearable biosensors for heavy metals analysis.

RESULTS AND DISCUSSION

The microsensors arrays are fabricated on a flexible polyethylene terephthalate (PET) substrate through a procedure involving multiple steps of photolithography, evaporation (Cr/Au, Ag, Bi), and lift-off as demonstrated in Figure 2. A 500 nm layer of parylene C is chosen as an insulation layer to ensure reliable measurement by preventing electrical contact of the conducting metal lines with body fluids and skin. Photolithography and O2 plasma etching are used to define the electrode area (100 μm × 1200 μm). After patterning the Ag reference, Bi and Au working electrodes, a thin Nafion coating is used as an antifouling layer to minimize biofouling of surface-active macromolecules such as proteins. A polydimethylsiloxane (PDMS) well (6 mm diameter and 1 mm thickness) was also bonded on top of the sensor array by soft lithography and O2 plasma etching. This ensures sweat accumulation (20–30 μL) such that a stable and reliable stripping analysis can be done. The microelectrodes array is then connected to a
Stripping conditions, same as Figure 3c. Insets in (c) and (d) show the corresponding temperature calibration plots of the sensors. The corresponding sensitivities for Zn, Cd, and Pb are 10.4, 7.1, and 5.4 nA/L/μg, respectively (Figure 3d). Note that high current at a very negative potential before the zinc peak is observed due to the hydrogen evolution. Figure 3e and 3f displays the linear response of a temperature sensor in physiological skin temperature range with a sensitivity of ∼0.24%/°C (normalized to the resistance at 20 °C).

The selectivity of the Bi and Au based microsensors are of great importance for the analysis in biofluids. Given the relatively high concentration of sweat Cu and Zn (on the order of hundreds μg/L), an interference study on Au and Bi based microsensors is implemented by varying Cu and Zn concentrations, respectively. As illustrated in Figure 4a, while the Cu concentration increases from 200 to 250 and 300 μg/L, the Cu current peak significantly increases while the current peaks of Pb and Hg (with a significantly lower concentration of 100 μg/L) remain unchanged. Similarly, while the Zn concentration is raised from 200 to 250 and 300 μg/L, the oxidation peak of Zn increases linearly with its concentration while there are no clear interferences on the peaks of Cd and Pb (with a concentration of 100 μg/L) (Figure 4b). Likewise, when varying concentrations of Cd, Pb, and Hg, no obvious interferences for the measurements of the other metals were observed on the Bi and Au microelectrodes. Such high selectivity of stripping analysis among the target heavy metals is crucial for the on-body test given the high complexity of human body fluids.

Another important aspect needed to be considered is the influence of temperature on the responses of biosensors. It has been reported that skin temperature and environmental temperature can have direct influence on metabolite sensors; hence, temperature compensation is essential to ensure accurate readings of the sensors. To this end, SWASV responses of the microsensors are investigated by gradually

As aforementioned, Au microelectrodes offer excellent biocompatibility and a wide operational potential window owing to their high stability. Au is an ideal electrode material for Pb, Cu, and Hg stripping. In order to investigate the relationship between the concentration of heavy metals and the response of SWASV, the voltammograms are recorded using a 0.01 M acetate buffer (pH 4.6) containing 50 mM NaCl and 100 μg/L Cu. Stripping conditions, same as Figure 3a. (d) Stripping voltammograms for Zn using a Bi microelectrode at physiological skin temperature range (20−40 °C) in 0.01 M acetate buffer (pH 4.6) containing 50 mM NaCl and 100 μg/L Zn. Stripping conditions, same as Figure 3c. Insets in (c) and (d) show the corresponding temperature calibration plots of the sensors.

Figure 4. Interference study and temperature compensation. (a) Interference study on a Au working electrode by varying Cu concentrations. Stripping conditions, same as Figure 3a. (b) Interference study on a Bi working electrode by varying Zn concentrations. Stripping conditions, same as Figure 3a. (c) Stripping voltammograms for Cu using a Au microelectrode at physiological skin temperature range (20−40 °C) in 0.01 M acetate buffer (pH 4.6) containing 50 mM NaCl and 100 μg/L Cu. Stripping conditions, same as Figure 3a. (d) Stripping voltammograms for Zn using a Bi microelectrode at physiological skin temperature range (20−40 °C) in 0.01 M acetate buffer (pH 4.6) containing 50 mM NaCl and 100 μg/L Zn. Stripping conditions, same as Figure 3c. Insets in (c) and (d) show the corresponding temperature calibration plots of the sensors.
increasing the temperature of the solution containing Cu(II) and Zn(II) from 20 to 40 °C. As shown in Figure 4c and d, responses of both Au and Bi electrodes increase almost linearly with increasing solution temperature over the range with a slope of 5.5%/°C and 6%/°C, respectively, relative to the peak height at 20 °C. The increase in the sensors’ responses reflects the elevated mass transport and electrochemical deposition and stripping kinetics. The integration of a temperature sensor into the microsensor array allows real-time temperature compensation to ensure an accurate and a reliable heavy metal detection.

The repeatability of Au and Bi based microsensors for stripping analysis of Cu and Zn was examined by recording the stripping voltammograms under the same condition mentioned above in a solution containing 150 μg/L Cu and Zn, respectively. Figure 5a and b shows 15 continuous stripping voltammograms and corresponding peak height for Cu detection using the same Au microelectrode. The relative standard deviation (RSD) of the peak measurements is 3.6%. Similarly, Figure 5c and d illustrates the stripping voltammograms and corresponding peak height for Zn detection using a Bi microelectrode with a 4.4% RSD for the peak height measurements. It should be noted that the Au-based sensors remain stable even after 100 times measurements while the performance of Bi-based sensors gradually decreases after 15 times test due to the consumption of Bi film. In addition, the reproducibility of different microsensor arrays for heavy metal analysis was examined. As demonstrated in Figure S2, the corresponding RSDs for Cu and Zn analysis using eight different microsensor arrays are 4.6% and 3.9%, respectively. Unlike the most common type of electroplated thick Bi film electrode, the Bi microelectrode displays excellent repeatability due to the high uniformity of the evaporated Bi film. It has been reported that the use of micro/nanoelectrodes can greatly enhance the mass transport rate due to small size. Additionally, the magnetic stirring commonly used for SWASV of regular macroscale electrodes to obtain repeatable and reliable data is avoided in our study. This represents a

Figure 5. Stability and repeatability of the microsensor arrays. (a,b) Stripping voltammograms (a) and corresponding peak height (b) continuously recorded with a gold microelectrode for 15 times in 0.01 M acetate buffer (pH 4.6) containing 50 mM NaCl and 150 μg/L Cu. Deposition at −0.7 V for 15 s; other stripping conditions, same as Figure 3a. (c,d) Stripping voltammograms (c) and corresponding peak height (b) continuously recorded with a bismuth microelectrode for 15 times in 0.01 M acetate buffer (pH 4.6) containing 50 mM NaCl and 150 μg/L Zn. Stripping conditions, same as Figure 3c. (e) Image of mechanical bending of a microsensor array (radii of curvature: 3.2 mm). (f) Peak height of the corresponding stripping results for 150 μg/L Cu and 150 μg/L Zn on Au and Bi based microsensors, respectively. Conditions, same as Figure 5a and b.
major advantage for the use of microelectrodes on wearable sensing devices.

The wearable microsensor array must be able to withstand mechanical deformation during vigorous physical exercise. The flexibility was investigated by monitoring the peak heights of stripping performance of the sensor array after mechanical bending (radii of curvature is 3.2 mm) (Figure 5e). As demonstrated in Figure 5f, no obvious variations (relative standard deviations are 1% and 1.9% for Cu and Zn detection, respectively) are observed for the stripping data even after 200 times bending tests. This indicates that the flexible microsensor array is robust enough for on-body test to endure the excess deformation during physical exercise.

A stable and a reliable performance of biosensors in biofluids is necessary for practical usage. To demonstrate such capability of the microsensor array (not only for on-body real-time monitoring but also for off-body analysis of different biofluids), sweat and urine samples are collected from volunteer subjects for off-body measurements. The physiological levels of all heavy metals in human sweat and urine are relatively low (<1 mg/L). Specifically, human sweat contains 100–1000 μg/L of Zn and Cu while the concentrations of Pb, Cd, and Hg usually fall below 100 μg/L. Using the microsensor array to analyze sweat and urine samples allowed visible oxidation peaks in sweat and urine samples of all the subjects. For Cu stripping, 15 s deposition time was used on the Au microelectrodes to minimize peak distortion and electrode fouling. In addition, the permselective/protective Nafion coating is found to be essential to address the challenge of biofouling due to the surface-active compounds in complex human biofluids. It helps to enhance oxidation peaks of targeted trace metals and allows direct detection in human sweat and urine samples. Cu and Zn levels in sweat (Figure S3a and b) and urine samples (Figure S3c and d) were determined by manual addition of target metal and back-calculating sweat concentrations as detailed in Experimental Section. Such a standard calibration method could minimize the potential influence of Cu–Zn intermetallic compounds formed on Bi electrodes (due to the high Cu levels in biofluids). Sweat samples were collected from subjects during a constant load indoor cycling exercise, while human urine samples were obtained from volunteer subjects. The measured concentrations of Cu and Zn in sweat and urine are consistent with earlier findings reported in the literature. Concentrations of Cu and Zn in biofluids were also validated with inductively coupled plasma mass spectrometry (ICP-MS). Table 1 illustrates the comparison between the measured concentrations of Cu and Zn in sweat and urine samples by using the microsensor array and by the ICP-MS method. No significant difference was observed between these two methods. This confirms that the microsensor array can be used for accurate measurement of heavy metals in sweat and urine. Although no obvious oxidation peaks of Pb, Cd, or Hg are observed due to their low concentration in sweat and urine of normal subjects, it is possible to detect them using the microelectrodes in those who are subjected to heavy exposure. It has been reported that Pb and Cd can reach 200–300 μg/L in sweat for the subjects undergoing heavy exposure. Figure 6 depicts the sensitivity of biosensors when heavy metals reach toxic levels in raw sweat (with the addition of 200 μg/L target analytes including Zn, Pb, and Cd).

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<th>Table 1. Comparison of the Measurement Results for Cu and Zn Content in Collected Human Sweat and Urine Samples from a Volunteer Subject Using the Microsensors and ICP-MS</th>
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Figure 6. Multiplexed heavy metal analysis in collected human sweat. Stripping voltammograms for detection of Zn, Cd, and Pb in a raw human sweat sample (collected from a subject during indoor cycling exercise) and in the same sweat sample with a standard addition of 200 μg/L of Zn, Cd, and Pb. Conditions, same as Figure 3c.

On-body heavy metals monitoring was performed during a constant-load exercise on a cycle ergometer. The protocol involved a 5 min ramp-up, 30 min cycling at 150 W, and a 5 min cool-down. The microsensor arrays were packaged in a wristband (Figure 7a) which could be comfortably worn by the subject. During the exercise, the skin temperature, sweat Cu, and sweat Zn were monitored by using an electrochemical potentiostat. Figure 7b and c shows the calibrated stripping voltammograms for heavy metal detection recorded by the microsensor array at different time during the exercise of a volunteer subject. The current readings were calibrated according to the real-time temperature information (stabilized at ~34 °C during the measurement period). It can be clearly seen that the initial concentrations of sweat copper and sweat zinc are high. As exercise continues, Cu and Zn concentrations decrease and gradually reach stable levels during the rest of the exercise. This general trend is also consistent with the ICP-MS data obtained from collected sweat samples in every 5 min period (Figure 7d). The slightly higher value from the on-body measurement is likely due to enhanced diffusion in the sweat sample during the deposition period caused by the subject’s vigorous motion.

CONCLUSIONS

In conclusion, we have developed a wearable and flexible microsensor array that can perform simultaneous and selective detection of multiple heavy metals (e.g., Zn, Cd, Pb, Cu, and Hg) noninvasively. The flexible microsensor arrays display very good repeatability and stability for heavy metal analysis. A temperature sensor is utilized for real-time compensation of the
signals to ensure accurate and reliable measurements. The microsensor array has been successfully used to accurately and selectively monitor heavy metal levels in human body fluids such as sweat and urine. Our device greatly expands the panel of analytes for noninvasive wearable biosensing. It could potentially be used to monitor heavy metal exposure and aid in related clinical investigations. Future work involves the full system integration of the flexible sensor array and the wearable circuit board for real-time data processing and transmission. Such a sensing platform is expected to significantly advance the field of wearable biosensors and to promote interdisciplinary collaborations toward realizing personalized medicine.

**EXPERIMENTAL SECTION**

**Materials.** Moisture-resistant polyethylene terephthalate (PET), 100 μm thick, was purchased from McMaster-Carr (Los Angeles, CA). Zinc, cadmium, lead, copper, and mercury standard AAS solutions (1000 mg/L in nitric acid), acetate buffer, sodium chloride (NaCl), and Nafion 117 solution (5 wt %) were purchased from Sigma-Aldrich (St. Louis, MO). All reagents were used as received.

**Fabrication of Electrode Arrays.** The fabrication process of the electrode arrays is illustrated in Figure 2. Briefly, the sensor arrays on PET were patterned by photolithography using positive photoresist (Shipley Microposit S1818) followed by 30/100 nm Cr/Au deposited via electron beam (e-beam) evaporation and lift-off in acetone. A 500 nm parylene C insulation layer was then deposited in a SCS Labcoter 2 Parylene Deposition System. Subsequently, photolithography was used to define the electrode area (100 μm × 1200 μm) followed by O2 plasma etching for 450 s at 300 W to completely remove parylene at the defined electrode areas. E-beam evaporation was performed to pattern 180 nm Ag on the electrode areas followed by lift-off in acetone. Finally, photolithography and e-beam evaporation were used to pattern 300 nm Bi on the electrode areas followed by lift-off in acetone. One microliter of Nafion 117 solution was then drop casted onto the microsensor array and dried for 2 h. A polydimethylsiloxane (PDMS) well (6 mm diameter and 1 mm thickness) was fabricated using a soft lithography process. It was bonded to the flexible PET substrate using O2 plasma etching treatment on the PDMS surface for 90 s at 90 W. The PDMS well allows the accumulation of sufficient sweat volume for stripping analysis. The microelectrodes array was connected to the potentiostat through an interface consisting of a flexible printed circuit connector.

**Characterizations of the Microsensor Arrays.** Square wave anodic stripping voltammetry (SWASV) was employed to characterize the electrochemical stripping of heavy metals on the microsensor arrays. In order to evaluate the performance of Bi electrodes, a deposition potential of −1.5 V (vs Ag+/Ag) was applied for 180 s, followed by a SWASV scan to a final potential of −0.5 V (vs Ag+/Ag) at a frequency of 60 Hz, an amplitude of 40 mV, and a potential step of 4 mV in 0.01 M acetate buffer (pH 4.6) containing 50 mM NaCl. In order to evaluate the performance of Au electrodes, a deposition potential of −0.7 V (vs Ag+/Ag) was applied for 120 s (15 s for repeatability tests and detection in biofluids), followed by a SWASV scan to a final potential of 0.8 V (vs Ag+/Ag) at a frequency of 60 Hz, an amplitude of 40 mV, and a potential step of 4 mV in 0.01 M acetate buffer (pH 4.6) containing 50 mM NaCl. Mechanical deformation was tested by repeatedly bending (radii of curvature is 3.2 mm) the microelectrodes array for 200 times.

**Off-Body Calibration and Validation in Human Sweat and Urine.** Sweat samples were collected directly from the forehead and urine samples were collected from the same volunteer subjects. And 50 μL sweat samples were collected from the same volunteer subjects. 50 μL sweat and urine samples were used for the off-body measurement. The level of heavy metals was estimated by SWASV through standard addition (1−2 μL each time) of 100 mg/L Zn or Cu standard solutions. It should be noted that, in some cases, the peak positions slightly shifted after the standard addition due to the greatly changed heavy metal concentrations. The measurement results of

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Figure 7. On body multiplexed trace metal detection using a microsensor array during a constant load (150 W) indoor cycling exercise. (a) Image of a microsensor array packaged in a transparent wristband. (b) On-body real time Zn analysis using a Bi based microsensor at different cycling time. Stripping conditions, same as Figure 5a. (c) On-body real time Cu analysis using a Au based microsensor at different cycling time. Stripping conditions, same as Figure 5c. (d) Calibrated sweat Cu and Zn levels measured real-time using a microsensor array during the exercise and measured off-body using ICP-MS from collected samples.
biofluid heavy metal levels from the microsensors were compared with the data measured directly through an ICP Optima 7000 DV instrument.

**Real Time On-Body Heavy Metal Analysis.** On-body evaluation of the microsensor arrays was performed in compliance with the protocol that was approved by the institutional review board at the University of California, Berkeley (CPSH 2014-08-6636). Five healthy subjects (all males), aged 20–40, were recruited from the University of California, Berkeley campus and the neighboring community through advertisement by posted notices, word of mouth, and email distribution. All subjects gave written, informed consent before participation in the study. An electronically braked leg-cycle ergometer (Kettler E3 Upright Ergometer Exercise Bike) was used for cycling trials. The subjects’ skin was cleaned with alcohol swabs and gauze before sensors were worn on-body. A constant workload cycling regimen was used in which subjects were cycling at 50 W with 50 W increments every 150 s up to 150 W, and then cycling at 150 W for 30 min. The 5 min cool down section involved cycling with decreased power output by 50 W every 150 s. During the exercise, on-body analysis was recorded using a Gamry electrochemical potentiostat (PCh4/G300). The on-body measurement results were calibrated using the measured skin/environment temperature at the same time. Such calibration eliminated the errors of stripping signals resulted from temperature variations. The heavy metal concentrations from on-body analysis was recorded using a Gamry electrochemical potentiostat (PCh4/G300). The on-body measurement results were calibrated using the measured skin/environment temperature at the same time. Such calibration eliminated the errors of stripping signals resulted from temperature variations. The heavy metal concentrations from on-body samples were roughly estimated using a coefficient factor obtained from an off-body standard addition method shown in Figure S3 (based on the sweat sample collected from previous running test of the same subject).

Three sweat samples were also collected in a 5 min period (10–15 min, 15–20 min, 20–25 min) followed by skin cleaning during the same exercise and tested with ICP-MS.

### ASSOCIATED CONTENT

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.6b00287.

Measurement of peak height from a stripping voltamogram, reproducibility of microsensor arrays, and heavy metal analysis in human body fluids (PDF)

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**Notes**

The authors declare no competing financial interest.

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Applications

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Human Sweat.


