



## Short communication

## Wearable temporary tattoo sensor for real-time trace metal monitoring in human sweat



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## ABSTRACT

A wearable electrochemical sensor for non-invasive monitoring of trace metals in human perspiration is described. The temporary tattoo-based printable stripping-voltammetric sensor has been applied for real-time monitoring of zinc in sweat using a bismuth/Nafion film electrode during physical activity. The Zn temporary tattoo sensor withstands repeated mechanical stress and displays a well-defined Zn response during on-body testing. Such a non-invasive stripping-voltammetric detection could be readily expanded to epidermal measurements of other relevant heavy metals.

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## 1. Introduction

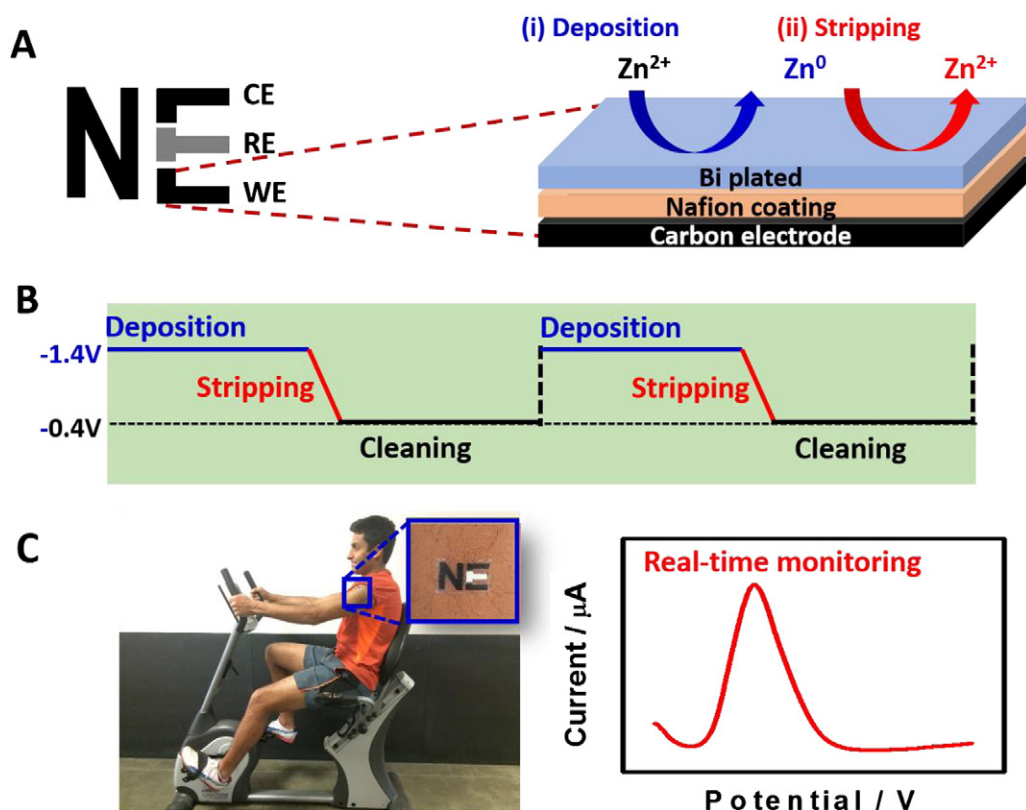
Wearable sensors can provide significant insights into the health status and performance of individuals [1]. Early efforts in this field have resulted in a variety of physical sensors to monitor activity and vital signs such as heart rate, skin temperature, respiration rate, or bodily motion [2,3]. Recently, our laboratory has extended these studies for non-invasive monitoring of electrolytes and metabolites on epidermis based on flexible temporary tattoo electrodes that adhere to the skin surface [4–8]. Such body-compliant printable electrochemical sensors offer elasticity characteristic of temporary tattoos along with resistance to mechanical stress and compatibility with the non-planarity of the epidermis. Expanding this attractive skin-worn platform towards new classes of analytes, in connection to different electrochemical techniques, should benefit diverse healthcare, fitness, and military applications.

In this article, we describe, for the first time, the fabrication and characterization of a wearable temporary tattoo sensor for the non-invasive monitoring of zinc, and trace elements in general, in sweat directly on the human epidermis (Fig. 1). Zinc is an important trace component of biochemical processes relevant to enzymes, hormones, and transcription-related factors [9]. Changes of the zinc concentration in bio-fluids can be used as indicators for wide ranging physiological states, such as muscular damage due to physical stress and the immune system [10–12]. Cordova et al. [13] observed that human subjects

experienced decreased endurance during physical activity associated with variation in zinc metabolism due to increased zinc excretion and stress. Perspiration represents an important pathway for the excretion of zinc, leading to its deficiency in the body [14]. While identifying the loss of zinc during physical activity is extremely important, currently no tools exist for real-time detecting of trace (physiological) levels of metals in sweat.

The stripping-voltammetric determination of Zn in sweat has been reported using collected sweat [15–17]. The use of collected sweat may be subject to inaccuracy due to sample contamination during collection, evaporation of sweat, and treatment steps. Additionally, it cannot yield real-time information on dynamic events. To address these limitations, we have combined in the present study, our expertise in fabricating skin-worn electrochemical temporary tattoo sensors [4–8,18], screen-printed electrodes for trace metal detection [19–21], and bismuth film electrodes for stripping-voltammetric metal measurements [22,23]. On-body electrochemical measurements of trace metals during exercise activity are thus demonstrated by implementing our printable temporary tattoo sensors directly on human skin. The sensor is prepared by modification of a flexible screen-printed electrode on a temporary tattoo-transfer paper with Nafion coating and ex situ plated bismuth film. The sensor response was characterized in buffer and on-body and tested under mechanical deformations associated with common physical activity. The Zn temporary tattoo sensor was applied successfully to several human subjects for direct epidermal metal sensing. Its attractive performance is discussed in the following sections.

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**Fig. 1.** (A) Schematic illustration of a temporary tattoo sensor and stripping-voltammetric analysis using an ex situ plated bismuth/Nafion working electrode. (B) Electrochemical procedure (time-potential cycle) of the stripping detection of Zn. (C) Real-time monitoring of Zn during cycling exercise with a temporary tattoo sensor transferred on a subject's deltoid.

## 2. Experimental

### 2.1. Reagents and instrumentation

All reagents were used as received. Acetate buffer, zinc, bismuth, lead, cadmium, and copper standard AAS solutions (1000 mg/L in nitric acid), sodium chloride, and Nafion® (5wt %) were purchased from Sigma-Aldrich (St. Louis, MO). Electrochemical measurements were performed using a  $\mu$ Autolab type II (Eco Chemie, The Netherlands).

### 2.2. Fabrication of temporary tattoo sensors

The NE temporary tattoo sensor design (Fig. 1A) is an abbreviation of the name of the department, “NanoEngineering”. The fabrication process of the screen-printed sensor on temporary transfer tattoo inkjet paper (HPS LLC, Rhine, TX) is similar to our previous work [8]. The patterned “E” portion of the temporary tattoo sensor design consisted of a pseudo-reference (Ag/AgCl), counter, and working electrodes (carbon ink). A transparent insulator was screen printed on the surface of the electrode pattern to confine the electrode and contact areas. Subsequently, the carbon working electrode was modified by drop-casting 2  $\mu\text{L}$  of Nafion®. After drying for 3 h, bismuth was electroplated at  $-0.8\text{ V}$  for 4 min using a 50  $\mu\text{g}/\text{mL}$  of bismuth solution (in 0.1 M acetate buffer).

### 2.3. Characterization of temporary tattoo sensors in buffer medium

Square wave anodic stripping voltammetry (SWASV) was employed to characterize the electrochemical stripping trace metal detection of the temporary tattoo sensor. In order to evaluate performance in buffer medium, sensors were transferred onto a plastic substrate for calibration and stability tests and onto a flexible GORE-TEX textile for mechanical strain tests. The operated SWASV is briefly depicted in Fig. 1B. A

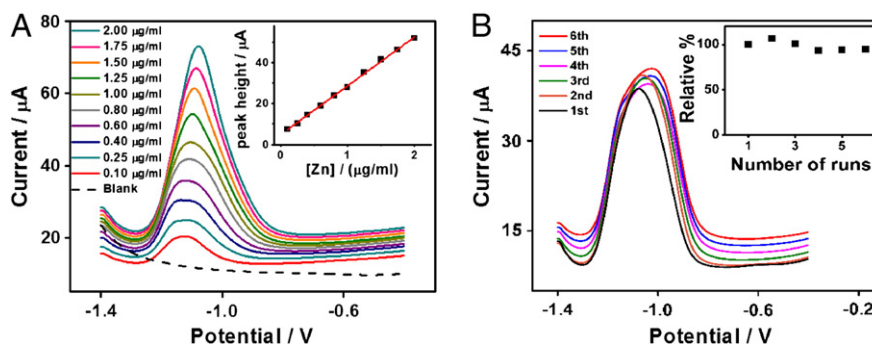
deposition potential of  $-1.4\text{ V}$  (vs. Ag/AgCl) was applied for 120 s, followed by a SWASV scan to a final potential of  $-0.4\text{ V}$  with a frequency of 25Hz, an amplitude of 25 mV, and a potential step of 4 mV in acetate buffer (pH 4.6) containing 0.1 M NaCl. A cleaning step followed every stripping step, a potential of  $-0.4\text{ V}$  was applied for 2 min to remove any remaining trace metals from the electrode surface. Sensors were calibrated using Zn solutions of 0.1–2.0  $\mu\text{g}/\text{mL}$  concentrations. Mechanical strain properties were tested by repeated 90° bending and stretching of the sensors up to 10% for 5 s on a GORE-TEX textile. This was done to mimic the viscoelastic properties of the skin. The sensors were subsequently relaxed for another 5 s and the procedure was repeated 5 times after which the SWASV response for 1  $\mu\text{g}/\text{mL}$  of Zn was measured.

### 2.4. On-body test of Zn temporary tattoo sensor

Epidermal studies were performed as described in our previous work [8], in strict compliance with the protocol that was approved by the institutional review board (IRB) at the University of California, San Diego. A total of 7 healthy volunteers (2 females and 5 males) were recruited. The temporary tattoo Zn sensor was transferred to a subject's arm in order to monitor the Zn concentration during exercise (Fig. 1C). Subjects were asked to ride a stationary cycle. They began cycling with a steady, comfortable cadence and continued with an intense cycling bout. During the exercise, SWASV on-body data were recorded with the  $\mu$ Autolab system. After the exercise, sweat was collected by using a pipette and the level of Zn was estimated by SWASV through standard addition of Zn solution.

## 3. Results and discussion

The Zn concentration in human perspiration depends on nutritional and health status of individuals. Lack of Zn causes subjects to feel

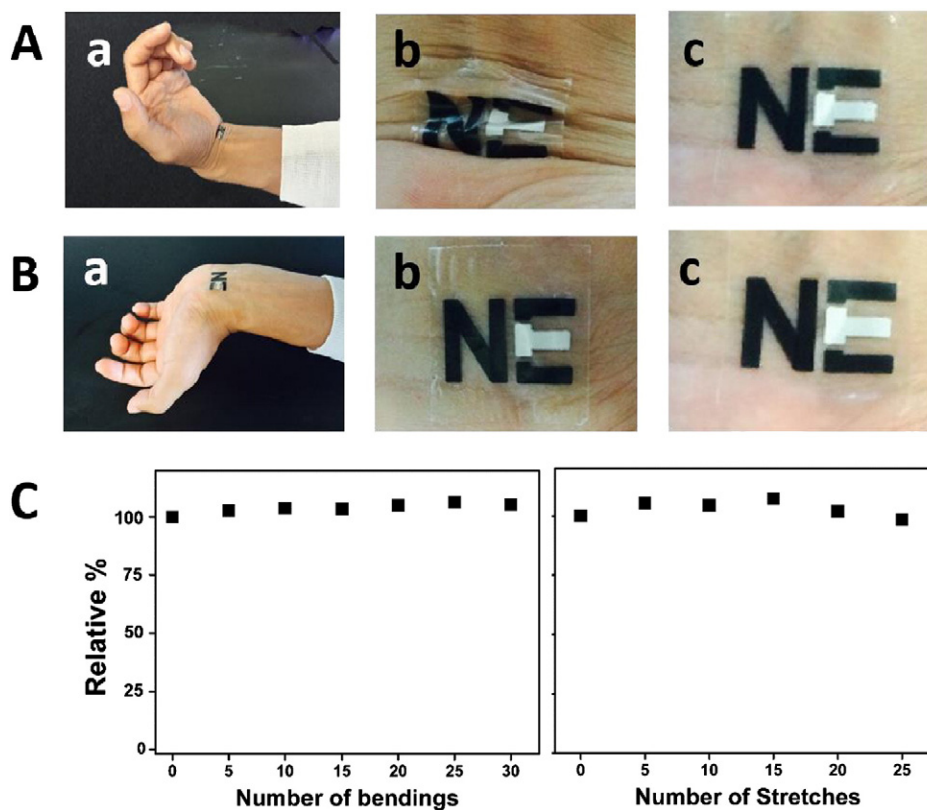


**Fig. 2.** Characterization of the printable temporary tattoo stripping metal sensor in buffer. (A) Stripping voltammograms for increasing Zn levels over a 0.1–2.0 µg/ml concentration range. The inset displays the corresponding calibration plot. (B) Stability of the response to 1 µg/ml Zn during 6 repetitive runs, along with the corresponding relative stripping peak response plot (inset). SWASV conditions: 0.1 M NaCl containing acetate buffer (pH 4.6); deposition potential at  $-1.4$  V for 120 s; SW stripping scan up to  $-0.4$  V using a frequency of 25 Hz, an amplitude of 25 mV, and a potential step of 4 mV. Each measurement was followed by a 2 min cleaning at  $-0.4$  V.

fatigued easily. The physiological level of Zn in human sweat is very low and ranges between 0.39 and 1.56 µg/ml [15,17,24]. In electrochemical detection, Zn deposits and strips at a very negative potential (close to the hydrogen evolution) compared to other trace metals. Further modification with bismuth and Nafion (Fig. 1A) was necessary to address the challenge of epidermal Zn monitoring. Bismuth electrodes are environmentally innocuous, simple to prepare and have higher sensitivity, well-defined peak current, large negative potential window and low sensitivity to dissolved oxygen, making them extremely useful for the trace metal electroanalysis [22,23,25]. An ex situ plated bismuth film electrode has been used due to its high stability. This has been coupled with a Nafion layer that facilitates the preconcentration of metal ions and minimizes biofouling [26,27]. It thus allows an enhanced peak response and direct operation in human sweat.

### 3.1. Characterization in buffer medium

To determine the performance of the Zn temporary tattoo sensor, SWASV was recorded in 100 µL acetate buffer (pH 4.6) containing 0.1 M NaCl with addition of Zn standard solution. NaCl was added to the buffer to simulate the composition of  $\text{Cl}^-$  in human sweat, which greatly improves the stripping signal of Zn, and will be referred to as acetate buffer in this paper [15]. Fig. 2A displays typical stripping voltammograms obtained at a bismuth/Nafion-modified temporary tattoo sensor with Zn concentrations increasing from 0.1 to 2.0 µg/ml, covering physiological levels of Zn in sweat. The bismuth/Nafion-modified temporary tattoo electrode offers a well-defined Zn stripping response following a 2 min deposition time. The anodic stripping peak appeared at around  $-1.1$  V vs. Ag/AgCl. A less negative oxidation peak of Zn is present at



**Fig. 3.** Photos of the “NE” temporary tattoo sensor on a human wrist for mechanical strain tests involving (A) bending and (B) stretching. (a) Bending and stretching of the wrist with the sensor, (b) the temporary tattoo sensor during bending and stretching tests, and (c) the sensor after 100 such bending and stretching tests. (C) Relative stripping peak response to 1 µg/ml Zn during a series of 30 such deformations, recorded after 5 such repeated bending (Left) stretching (Right) tests. SWASV parameters, as in Fig. 2.

equilibrium between Zn(II) and  $\text{Cl}^-$ , which also improves the Zn oxidation current [15]. The Zn peak current increases proportionally to Zn concentration showing a highly linear calibration plot with a slope of  $23.8 \mu\text{A}\cdot\text{ml}/\mu\text{g}$  ( $R^2 = 0.999$ ) (Fig. 2A, inset). Furthermore, a detection limit of around  $0.05 \mu\text{g}/\text{ml}$  can be estimated on the basis of the signal-to-noise characteristics ( $S/N = 3$ ) of these data. Subsequently, the repeatability of the temporary tattoo Zn sensor was examined using a  $1 \mu\text{g}/\text{ml}$  Zn solution (Fig. 2B). After 6 repeated measurements, 94.7% of signal was retained, reflecting the reproducibility and stability of the temporary tattoo Zn sensor response (RSD of 5.2%).

Potential epidermal monitoring during a variety of physical activities requires an assessment of the sensor's resilience against mechanical deformations experienced during such on-body applications. The temporary tattoo Zn sensor was initially transferred on a human wrist, where it can experience large and frequent movements (Fig. 3A, B(a)). It underwent repeated bending and stretching for 100 times to mimic severe mechanical stresses that may occur during physical activity. No observable damage to the temporary tattoo electrode after these stress cycles was noted (Fig. 3A, B). Furthermore, the electrochemical response was retained under 30 such repeated mechanical stress cycles, reflecting the robustness of the sensor to endure deformation stress during exercise (Fig. 3C).

### 3.2. On-body characterization of the Zn temporary tattoo sensor

The on-body monitoring performance of the Zn temporary tattoo sensor was evaluated during physical activity. The temporary tattoo sensor was transferred to the deltoid of participants (Fig. 1C), based on previously reported procedures [7,8]. During each test, the volunteer cycled for 15–20 min to generate a visible amount of sweat on their skin; this enabled completion of the “electrochemical cell” essential for Zn detection with a stable background current and a well-defined stripping peak. Fig. 4 shows representative voltammograms from three subjects during physical activity (A–C). These results show well-

defined Zn stripping peaks around  $-1.15 \text{ V}$ , indicating that the wearable Zn temporary tattoo sensor can successfully detect the presence of Zn in the sweat during exercise. The voltammogram of subject A shows an additional small peak around  $-0.6 \text{ V}$  (Fig. 4A), attributed to the presence of Pb in sweat, and reflects the multi-element sensing capability of stripping voltammetry. The stripping behavior is only slightly compromised by the on-body operation (vs. the in buffer data of Fig. 2), leading to a broader peak, higher background current and small shoulders. The latter is attributed to the formation of Zn-Cu intermetallic compound, reflecting the high Cu sweat levels ( $0.4 \mu\text{M}$ – $33.2 \mu\text{M}$ ) [28]. Finally, to quantify the amount of Zn in sweat, subject C's sweat was collected and analyzed. Zn standard additions were successively applied for off-body stripping analysis of this untreated sweat sample (Fig. 4D). The corresponding linear standard addition plot ( $R^2 = 0.996$ ) results in a zinc sweat concentration value of  $0.34 \mu\text{g}/\text{ml}$ , which is consistent with the reported range between  $0.39$  and  $1.56 \mu\text{g}/\text{ml}$  [15]. This wide Zn concentration range in sweat can be attributed to differences in the daily uptake of food, beverages, medication, and health status along with other environmental effects.

### 4. Conclusions

We have demonstrated the first example of a wearable electrochemical trace metal sensor. This represents the first example of on-body non-invasive measurements of trace elements. The new temporary tattoo-based printable stripping-voltammetric sensor has been used for real-time monitoring of Zn in human perspiration using a bismuth/Nafion-coated electrode. The Zn temporary tattoo sensor withstands repeated mechanical stress fatigues and displays a well-defined and stable Zn response in human sweat during on-body testing. Such stripping-based epidermal sensor could be readily expanded to non-invasive monitoring of other relevant heavy metals. Future efforts towards such trace metal monitoring will focus on miniaturization and integration of electronics for data acquisition, processing, and wireless transmission.

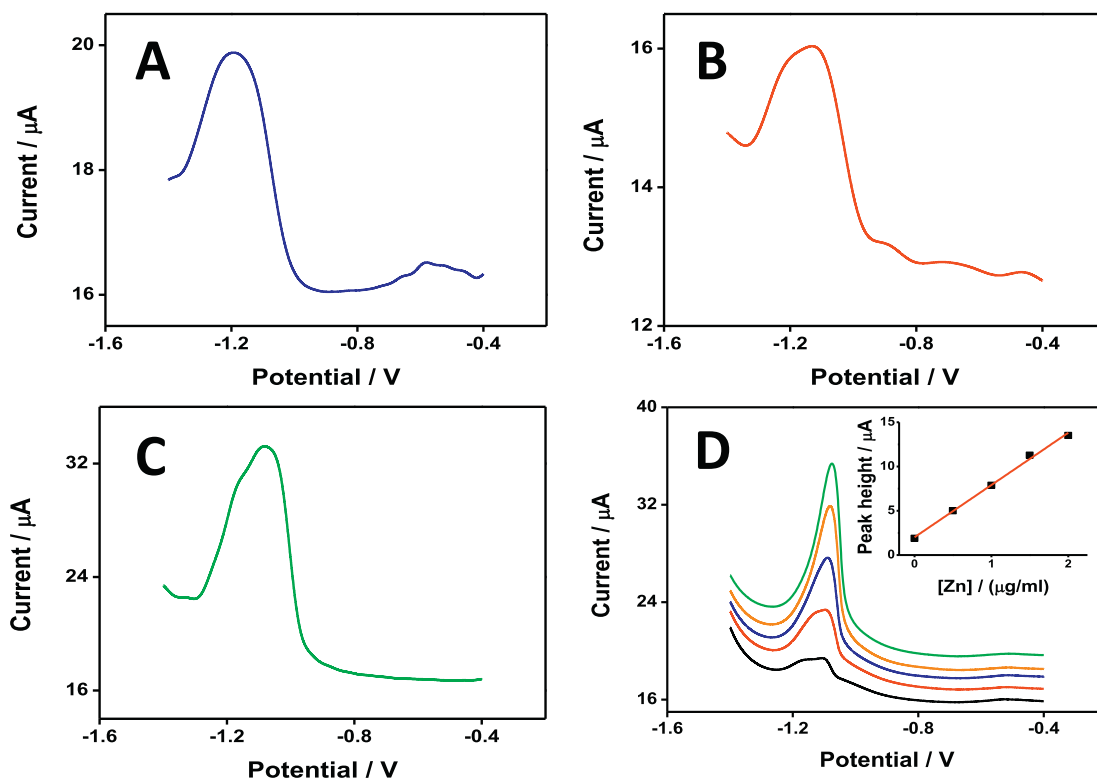


Fig. 4. (A–C) On-body SWASV results obtained for real-time monitoring of Zn in sweat during the cycling exercise for three different human subjects. (D) SWASV result for successive standard additions of Zn, recorded with the temporary tattoo sensor using human sweat samples (collected from subject C). SWASV parameters, as in Fig. 2.

## Conflict of interest

The authors declare no conflict of interest.

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