

A Wearable Nutrition Tracker

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Nutrients are essential for the healthy development and proper maintenance of body functions in humans. For adequate nourishment, it is important to keep track of nutrients level in the body, apart from consuming sufficient nutrition that is in line with dietary guidelines. Sweat, which contains rich chemical information, is an attractive biofluid for routine non-invasive assessment of nutrient levels. Herein, a wearable sensor that can selectively measure vitamin C concentration in biofluids, including sweat, urine, and blood is developed. Detection through an electrochemical sensor modified with Au nanostructures, LiClO₄-doped conductive polymer, and an enzymes-immobilized membrane is utilized to achieve wide detection linearity, high selectivity, and long-term stability. The sensor allows monitoring of temporal changes in vitamin C levels. The effect of vitamin C intake on the sweat and urine profile is explored by monitoring concentration changes upon consuming different amounts of vitamin C. A longitudinal study of sweat's and urine's vitamin C correlation with blood is performed on two individuals. The results suggest that sweat and urine analysis can be a promising method to routinely monitor nutrition through the sweat sensor and that this sensor can facilitate applications such as nutritional screening and dietary intervention.

Nutrition is a critical factor that modulates our physiological functions throughout our lives.^[1,2] Nutritional imbalance can affect fetal, infant, and maternal health and can result in low birth weight and birth defects of the brain and spine.^[3] In children and adolescents, poor nutrition can cause failure to thrive


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and have long-term effect on physical and mental development.^[4] Malnutrition is also a prevalent problem in hospitalized patients and institutionalized elderly people,^[5,6] leading to enhanced morbidity and making disease recovery difficult.^[5] Besides, over supplementation of nutrients can have toxic effects such as obesity and metabolic disorders. Dietary guidelines can generally apply to healthy individuals, but biological and physiological changes in certain groups such as elders, pregnant women, and critically ill patients require necessary adjustment for effective nutrient absorption.^[7] Therefore, it is important to periodically monitor nutrients within the body to prevent adverse effects of malnutrition. Traditionally, nutritional assessments have been done based on dietary, clinical, and anthropometric assessments, universal screening tools, and serum biomarkers.^[8] Yet they require professional involvement, patient's food and health history, and hence, cannot provide quick

assessment for dietary intervention. Incorporating a measurement tool that allows periodic monitoring of nutritional status may help assessment and make intervention less demanding.

Sweat contains a variety of biochemicals that can be indicative of underlying physiological conditions.^[9,10] Sweat has traditionally been utilized for medical diagnosis of cystic fibrosis in the hospitals and for drug abuse in forensic toxicology.^[11,12] A rapid rise in wearable sweat analytical devices in recent years brings promising new applications for sweat in health care.^[13–22] For instance, sweat ethanol level is correlated with blood ethanol, and uric acid in sweat can reflect plasma uric acid level in gout patients.^[23,24] These discoveries guide the possibility of sweat analysis in wider applications such as in nutritional assessment. Studies have shown that sweat composition includes nutrients such as vitamins, amino acids, proteins, and carbohydrates.^[25] A recent work on a wearable vitamin C sensor showed that dynamic change in sweat could be detected as vitamin C was consumed.^[26] Therefore, studying sweat dynamics in response to changes in nutritional implications may help expand the value of sweat in nutritional testing. Developing a wearable device that provides quick measurement of nutrition status within the body may enhance health management of individuals.

Herein, we developed a wearable sensor that allows monitoring of nutrients such as vitamin C in biofluids, including sweat, urine, and blood. Vitamin C quickly oxidizes under

atmospheric conditions^[27] and hence requires rapid detection upon secretion for accurate analysis of its concentration. Our sensor allows such capability to facilitate periodic nutritional analysis. The sensor utilized poly(ethylene terephthalate) (PET) as a flexible substrate for conformal contact with the skin. We enable detection of vitamin C from μM to mM level through simple surface modifications with Au nanodendrites, electroactive conducting polymer, and protective membrane layer such that sensors can selectively and stably measure target analyte regardless of biofluid compositions. We monitored its concentration in sweat along with urine and blood of different subjects to explore changes in biofluids in response to oral intake of vitamin C. Our study shows that increasing consumption of vitamin C from 0 to 1000 mg raises its concentration in sweat. Urine-vitamin C concentration generally increases with increasing intake when water intake is controlled. By comparing sweat- and urine-vitamin C levels to blood for two individuals, we found a correlation coefficient of 0.81 and 0.72, respectively. Our findings suggest that sweat and urine can be promising biofluids to monitor nutrition regularly and can assist clinical evaluation of nutritional status. The results also suggest that it may be promising to explore a wide range of targets for a more comprehensive nutritional screening to help with the better management of an individual's well-being.

Our sensor utilizes a standard three-electrode system in which the working, counter, and reference electrodes lie on a planar flexible substrate. The electrodes are fabricated through photolithography and evaporation on a thin PET with a thickness of 100 μm . The surface of the vitamin C selective electrode

is modified with multiple layers as shown in Figure 1a to achieve reliable detection in biofluids. The electrode surface contains Au nanodendrites to enhance measured signal and better attachment of enzymatic membrane to the conductive surface.^[28] The electrochemically deposited conducting polymer poly(3,4-ethylenedioxythiophene) doped with lithium perchlorate (PEDOT:LiClO₄) has been shown to have superior electrochemical properties such as high charge injection limit and stable electroactivity.^[29] It has previously been utilized in functionalization of vitamin C sensor along with carbon nanotubes on saturated calomel electrode.^[30] Encapsulation of the enzyme-immobilized surface with Nafion further promotes the sensor's long-term usage and antifouling in biofluids.^[22] The reference electrode contains an Ag/AgCl layer, and planar Au is utilized as the counter electrode. The detection is based on oxidation of vitamin C (ascorbic acid) with L-ascorbate oxidase as shown in Figure 1b. The sensor is flexible and can have conformal contact with the skin through a thin water absorbent pad (Figure 1c) to assist absorption of biofluids onto the sensor. The sensor requires a minimum $\approx 1.5 \mu\text{L}$ of fluid volume on the absorbent pad for a reliable measurement. Hence, for typical sweat rates stimulated by iontophoresis ($>100 \text{ nL min}^{-1} \text{ cm}^{-2}$), one measurement can be done in ca. $<75 \text{ min}$. For blood measurement, a single drop of blood is sufficient for one measurement. Figure 1d shows a conceptual idea of the potential application of the sensor. By integration of the electronic circuitry for wireless monitoring, as shown similarly in our prior works,^[13] users can be informed of the nutrition status so that proper measures can be taken to keep track of nutritional balance periodically.

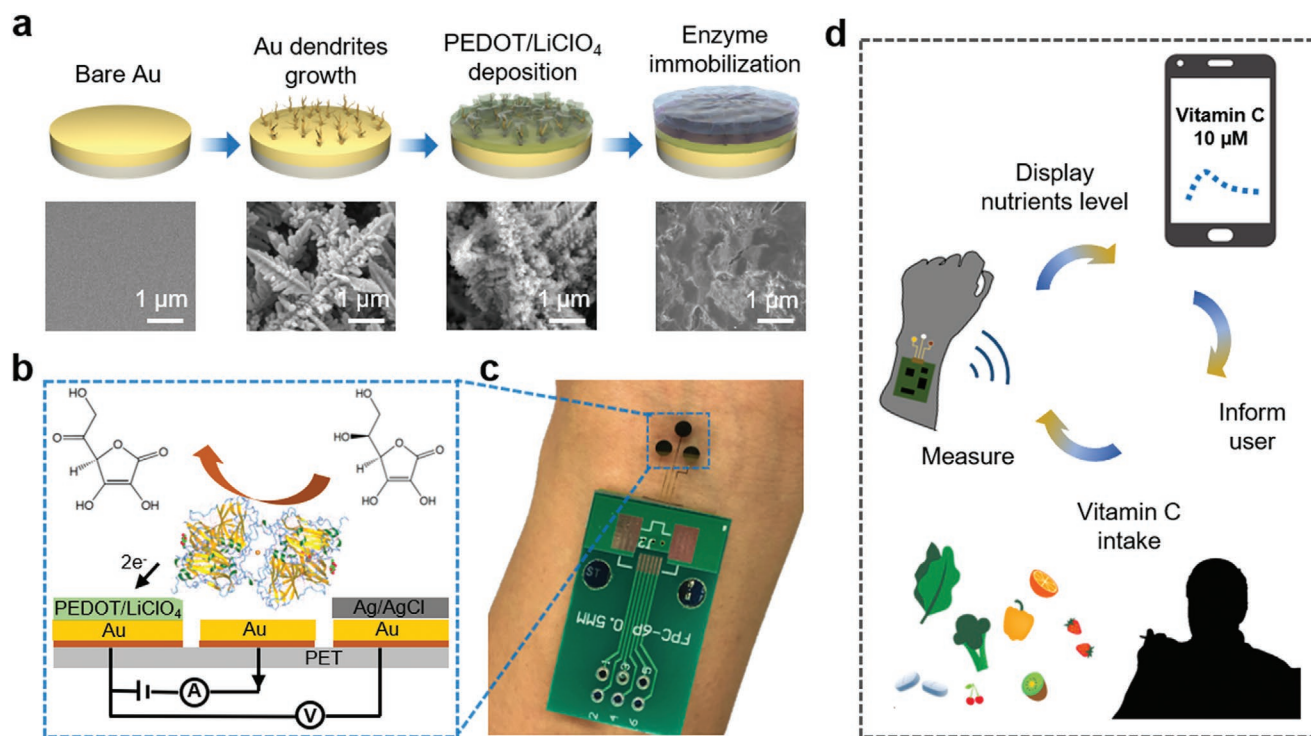


Figure 1. Schematic of the wearable vitamin C sensor for nutritional assessment. a) Illustration showing the fabrication procedures of vitamin C sensor and corresponding SEM images. b) Circuit showing the sensing process of the vitamin C sensor. c) Sensor on a user. d) Schematic showing the prospective application of the vitamin C sensor.

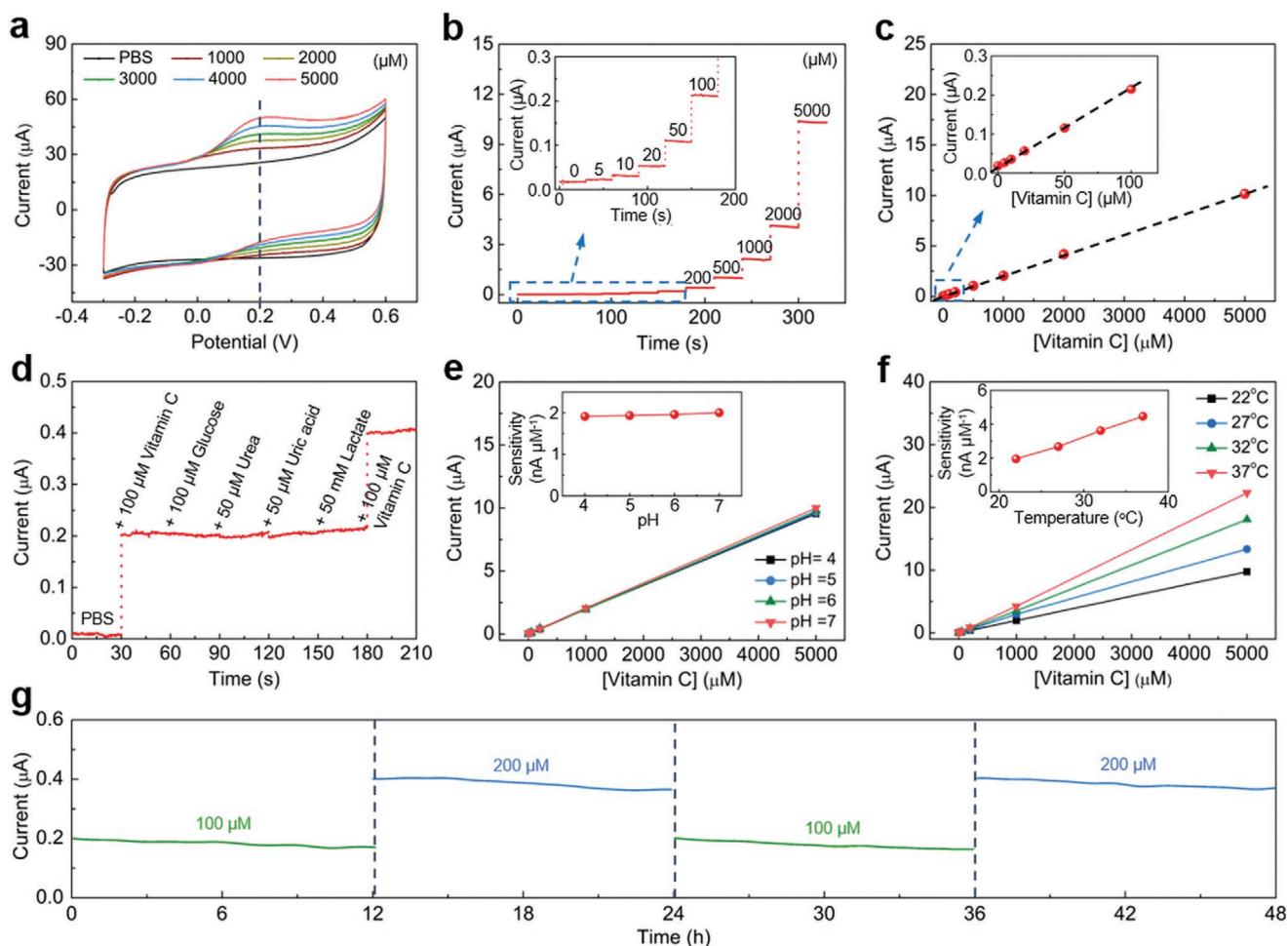


Figure 2. Performance evaluation of the vitamin C sensor. a) CV curves of the vitamin C sensor in different concentrations (units are in μM) of vitamin C at a scan rate of 50 mV s^{-1} . b) Amperometric response of the sensor. Measurements were paused for 2 min while changing concentrations. c) Sensitivity of the vitamin C sensor (Insets show the sensor's response in low vitamin C concentration range). d) Selectivity test of the sensor. e) pH and f) temperature effects on the sensor performance (Insets show sensitivity of the sensor at different pH and temperatures). g) 48 h long-term measurement of the vitamin C sensor. Measurements were paused for 2 min while changing concentrations.

First, to investigate the influence of Au nanotextures on the sensor performance, we performed cyclic voltammetry (CV) by scanning the sensors functionalized on bare Au electrode and Au nanotextured electrode from -0.3 to 0.6 V in 5 mM vitamin C solution. The measured results in Figure S1a, Supporting Information, show that the nanotextured electrode has a more distinct oxidation peak of ascorbic acid at $\approx 0.2\text{ V}$. The thin layer of PEDOT:LiClO₄ with outward-projecting dendritic structures (Figure 1a) assist electron transfer and increase the electroactive area, hence, improving the current signals compared to the sensor fabricated on a planar surface. The peak becomes more prominent with increasing vitamin C concentration from 1000 to $5000\text{ }\mu\text{M}$ vitamin C as displayed in Figure 2a. This oxidation voltage is lower than the majority of the similar vitamin C sensors reported in the literature.^[30–32] A wearable sensor developed on a rhodium–carbon electrode for chronoamperometric measurements required ca. -0.5 V for vitamin C detection.^[26] The lower oxidation voltage in this work is due to the enhanced electroactive mediator PEDOT:LiClO₄,^[28] which can assist catalysis of the oxidation of ascorbic acid at 0.2 V . This voltage was used

for subsequent characterizations of the sensor and measurement of vitamin C concentrations in biofluids. A healthy person typically contains $1\text{--}37\text{ }\mu\text{M}$ vitamin C in sweat,^[25] $0\text{--}2000\text{ }\mu\text{M}$ in urine,^[33] and $\approx 10\text{--}200\text{ }\mu\text{M}$ in plasma depending on the oral dosage of vitamin C.^[34] Therefore, the sensor was characterized by measuring amperometric responses to change in concentrations from 0 to $5000\text{ }\mu\text{M}$ as presented in Figures S1b and S2b, Supporting Information. The sensor showed a linear response within the measured range (Figure 2b,c) with a sensitivity of 1.2 and $2.0\text{ nA }\mu\text{M}^{-1}$ for bare Au and nanotextured electrode, respectively. Higher sensitivity of the nanotextured electrode is due to higher surface area available for efficient electron transfer. The sensor's response time was determined to be $\approx 35\text{ s}$ based on the amperometric curves to reach within 10% variation from the stable current. This process is shown in Figure S2, Supporting Information. To show the reproducibility across sensors, we also studied sensitivity variation in five different sensors. The results displayed in Figure S3, Supporting Information, indicates a sensitivity variation of $\approx 12\%$ for vitamin C ranging from 0 to $5000\text{ }\mu\text{M}$. This variation is smaller than typical vitamin C

concentration changes throughout the day;^[35] however, sensors' signal can be calibrated prior to measurement for higher measurement accuracy. Based on Figure S3, Supporting Information, the limit of detection (LOD) was found to be $\approx 4 \mu\text{M}$, which is above the common vitamin C concentrations found in biofluids. The LOD was computed by taking the average sensitivity and the standard deviation (STD) of the five sensors and computing $3 \times \text{STD}/\text{sensitivity}$.

The sensor was further tested to investigate its selectivity against various metabolites which may interfere with the vitamin C signals. Figure 2d shows that the sensor is very selective to the target with negligible influence from foreign chemicals like glucose, urea, uric acid, and lactate. As the sensor is based on enzymatic reactions, it may be prone to pH changes in the solution. Therefore, the sensor's performance was evaluated in buffer with pH 4, 5, 6, and 7. As displayed in Figure 2e, the sensitivity of the sensor slightly increases with increasing pH. However, such an increase is small compared to the sensitivity of the sensor; hence, pH influence is not considered in biofluid measurements. The sensor's response to temperature variation was additionally explored in Figure 2f. The sensitivity of the sensor increases as the test solution's temperature is raised from 22 to 37 °C. As our subject studies were performed under controlled room temperature, for each individual, we assumed the skin temperature remains relatively constant. Finally, the sensor was tested for 48 h to show its capability for long-term usage. Figure 2g shows that the measured current recovers as vitamin C concentration is switched between 100 to 200 μM in 48-h duration. Further, the stability of the sensor for long-term storage was also explored as presented in Figure S4, Supporting Information. Based on the result, it is clear that the sensitivity variation of a single sensor across 5 days is less than 2%. Such long-term stability for monitoring and storage is possible due to the Nafion layer which hinders enzymes escaping from the sensing surface.^[36] These results confirm that the sensor can be reliably used for measuring vitamin C concentrations in biofluids. Last, we performed mechanical tests on the sensors to show its usage as flexible sensors on skin. Figure S5a, Supporting Information, shows that after 0, 50, and 100 bending cycles on 20 and 80 μM solution, the current signals remain nearly the same. While the sensor was bent or unbent, the current remains constant as shown in Figure S5b, Supporting Information. Therefore, the sensors are suitable for wearing on skin.

To explore sweat capability in reflecting vitamin C consumption, we first investigated the behaviors of sweat, along with urine, to an oral intake of 1000 mg vitamin C. Sweat was induced by iontophoresis in the morning under fasting condition and 3 h after vitamin C intake. 3 h was chosen because blood vitamin C level reaches its maximum between 2–5 h after the oral dosage.^[35] Subjects were asked to refrain from alcohol and caffeine intake a day prior to the trial. Figure 3a–l shows results obtained from a series of trials on six subjects. To ensure reproducibility of the results, we performed four trials on subjects 1 and 2 separately on different days. The amount of water intake during the measurement period was not controlled in these trials. In these six subjects, we found that the fasting vitamin C concentration in sweat is ≈ 5 –20 μM with increase in concentration of 5–15 μM after the oral intake. On

the other hand, urine has a fasting concentration of 10–250 μM with increase in 1.5–5 mM after the oral dosage. Subjects 1 and 2 consistently show an increase in sweat- and urine-vitamin C concentrations after the oral intake in all repeated trials. The concentration ranges found in the trials are consistent with the reported values in the literature.^[25,33,35,37] Based on the results, the vitamin C levels in both sweat and urine rise after the oral intake; however, the percentage change in concentration is not the same for each trial.

To realize the potential of sweat and urine to periodically monitor vitamin C inside the body, we conducted monitoring of sweat and urine in two subjects under controlled water intake for 2 days. While there is limited understanding of the dynamics of sweat nutritional composition, urinalysis for nutrition were traditionally conducted by monitoring 24 h urine samples.^[35] Hence, it is important to explore temporal changes of nutritional compositions in sweat and urine. A general timeline of the study is displayed in Figure 4a. We monitored the concentrations in sweat induced from both left and right hands of the subjects to account for the possible variation between different sides of the body. Detailed procedure is described in the Experimental Section. Figure 4b,c shows measurement results obtained from two subjects. Both subjects initially had low baseline vitamin C levels ($<10 \mu\text{M}$) in sweat and ($<200 \mu\text{M}$) urine and quickly increased by at least 2 and 20 times, respectively, 3 h after 1000 mg intake of vitamin C. 8 h after the intake, the urine-vitamin C is slightly lowered from its maximum but remains higher than the baseline for both subjects. However, the sweat-vitamin C level reaches nearly the same concentration with baseline for subject 2. The same behavior is discovered on the 2nd day. Despite the difference in the left and right hands' vitamin C concentrations, we observe the same trend across 2 days. These behaviors are in fact similar to the pharmacokinetic profile of plasma vitamin C after oral dosage.^[34,35] We additionally performed tests for three consecutive days, in which in the first 2 days no significant vitamin C consumption was there and in the last day there was a 1000 mg oral intake, on a single subject as shown in Figure S6, Supporting Information. We observed relatively low vitamin C concentrations in both sweat and urine on the first 2 days and an abrupt increase in both biofluids after 1000 mg intake on the 3rd day. The result is similar to those displayed in Figure 4.

Finally, to further investigate how sweat- and urine-vitamin C concentrations changes with varying oral dosages, we performed sweat and urine analysis on a single individual under 0, 500, 1000, and 2000 mg of vitamin C intake (Figure S7a–d, Supporting Information). In these trials, we controlled the subject's water intake to ensure the water intake is the same during the measurement duration. Each measurement was done a week apart. Blood measurements were taken right after sweat measurement. In general, the baseline of vitamin C in sweat and urine differ from trial to trial (average concentration of 7–12 μM in sweat and 10–200 μM in urine). When the results collected at 3 h after oral intake is plotted against the dosage amount, we found that sweat- and urine-vitamin C level rises with increasing intake of vitamin C. Specifically, sweat-vitamin C becomes higher with increasing dosage from 0 to 1000 mg; however, sweat-vitamin C concentration does not rise further upon increasing the dosage to 2000 mg (Figure 5a). On

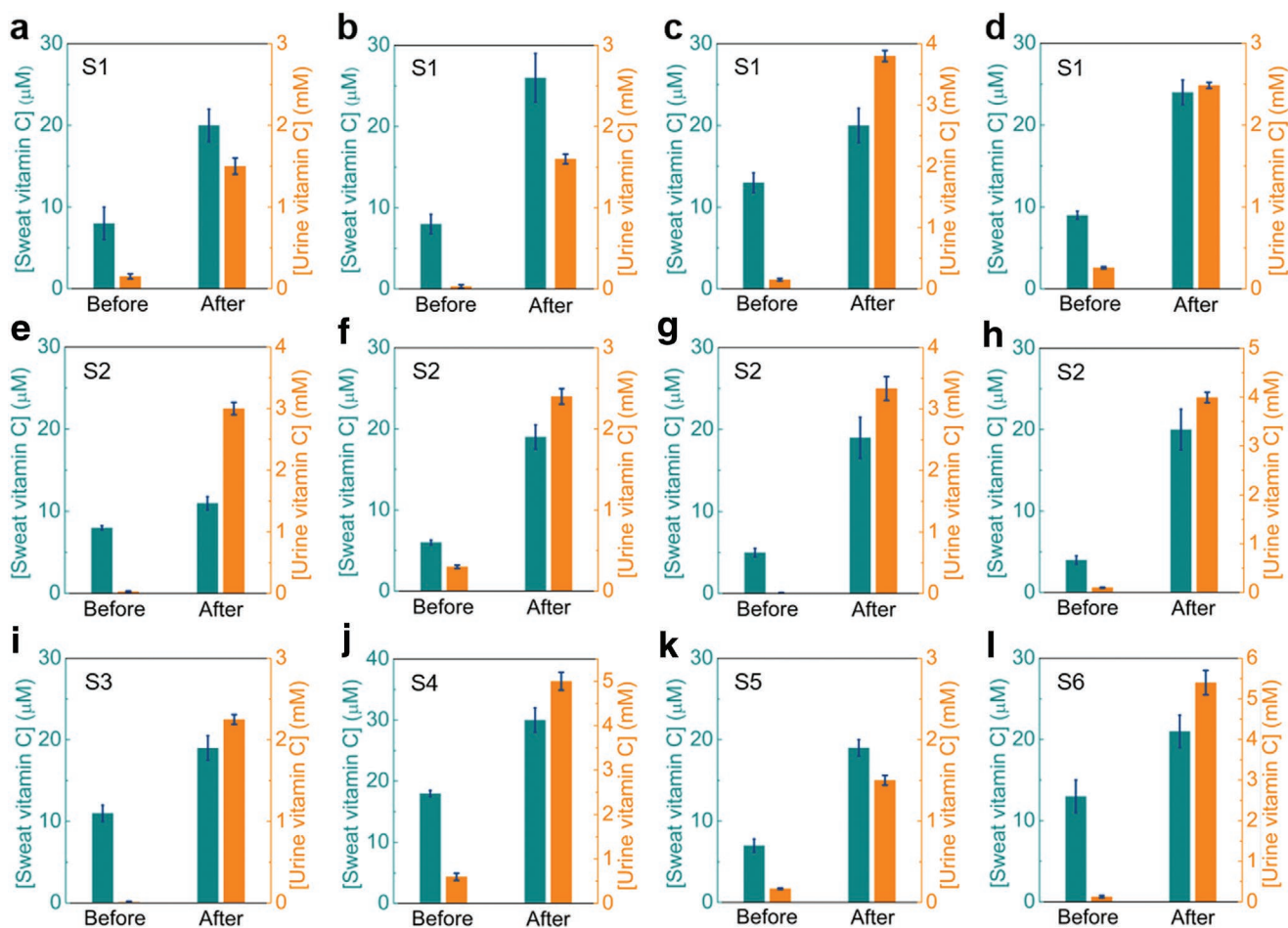


Figure 3. Comparison of the sweat- and urine-vitamin C levels of six subjects before and after 1000 mg vitamin C intake. a–d) 4 trials for subject 1 (S1). e–h) 4 trials for subject 2 (S2). i–l) Subjects 3–6 (S3–S6). The standard deviations indicated in the figures are obtained from three measurements for each test.

the other hand, urine-vitamin C increases abruptly from 0 to 500 mg dosage and rises slowly from 500 to 2000 mg as shown in Figure 5b. This behavior is similar to the 24-h measurement of total amount of urine-vitamin C.^[35] Moreover, we also compared sweat, urine, and blood concentrations to determine if correlation can be constructed. By plotting the sweat and urine together with the blood tests' results obtained from the two individuals, we found that both sweat and urine levels increase with increasing blood vitamin C as presented in Figure 5c,d. Based on the result, sweat and blood has a correlation coefficient of 0.81 and urine and blood has 0.72. These results provide promising examples to assess nutrition information within the body.

In summary, we developed a wearable sensor that allows reliable detection of vitamin C concentration in sweat, urine, and blood. By utilizing the sensor, we are able to monitor sweat and urine profile upon vitamin C consumption. Our major finding includes that sweat and urine can be related to blood vitamin C and may serve as ideal candidates for routine assessment of nutritional health in general population to hospitalized patients. Although the water intake is controlled to minimize variation in urinary analysis, our finding on urine relation to blood and

vitamin C consumption bring promising applications for future urine testing. Moving forward, a more comprehensive nutritional analysis with clinical nutritional assessments will facilitate better understanding of the importance of sweat. We envision that our sweat biosensor could be a promising tool for providing guidance of daily diet and realizing personalized health management.

Experimental Section

Materials: Hydrochloric acid (HCl), chloroauric acid (HAuCl_4), Nafion 117, 3,4-ethylenedioxythiophene (EDOT), lithium perchlorate trihydrate ($\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$), vitamin C, and ascorbate oxidase (from *Cucurbita* sp., 650 U g^{-1}) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Silver ink CI-4040 was purchased from EMS Adhesives.

Sensor Fabrication: The electrodes for sensor functionalization were fabricated on a flexible PET substrate using traditional photolithography and evaporation methods. Briefly, the PET substrate was cleaned with isopropyl alcohol and O_2 plasma, and the electrodes with a diameter of 3 mm were patterned via photolithography and thermally evaporated with 30/50 nm of Cr/Au, followed by lift-off in acetone. Au nanostructures were electrochemically deposited on a bare Au electrode using a solution containing 50 mM HAuCl_4 and 50 mM HCl. The deposition was

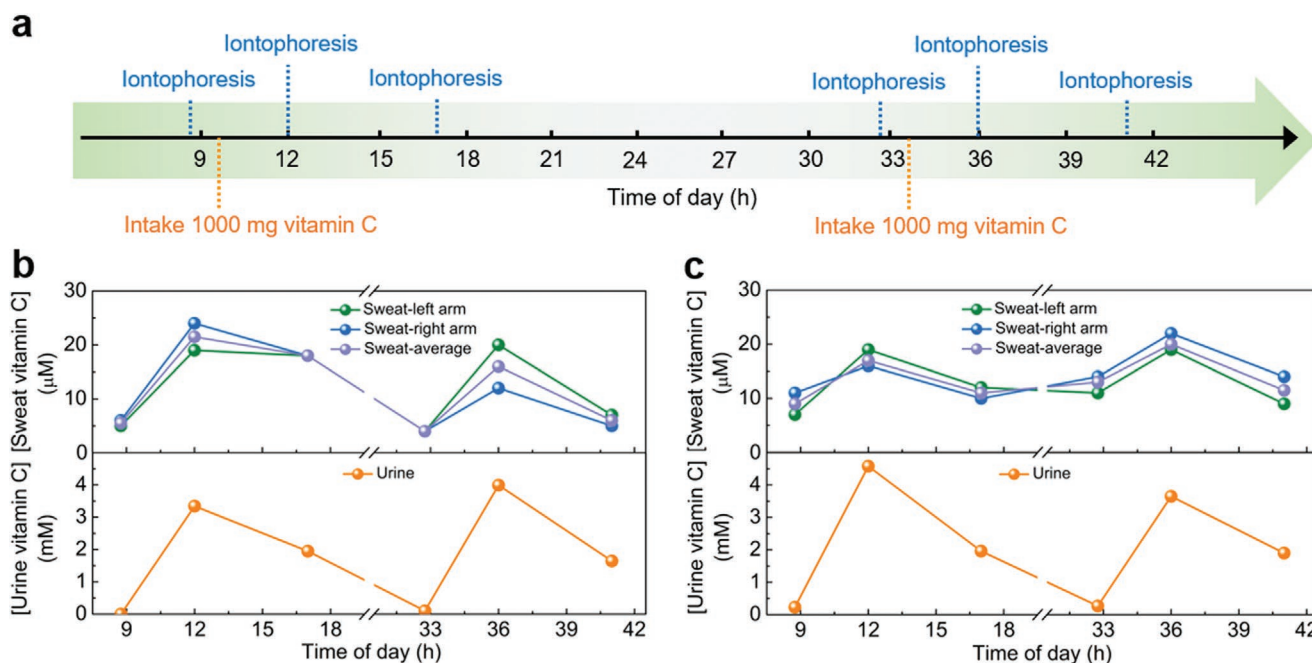


Figure 4. Vitamin C monitoring in two consecutive days. a) A timeline for 48-h subject studies where subjects consumed 1000 mg of vitamin C on each day is shown. b, c) Temporal changes of the sweat- and urine-vitamin C levels in subject 1 (b) and subject 2 (c) are displayed.

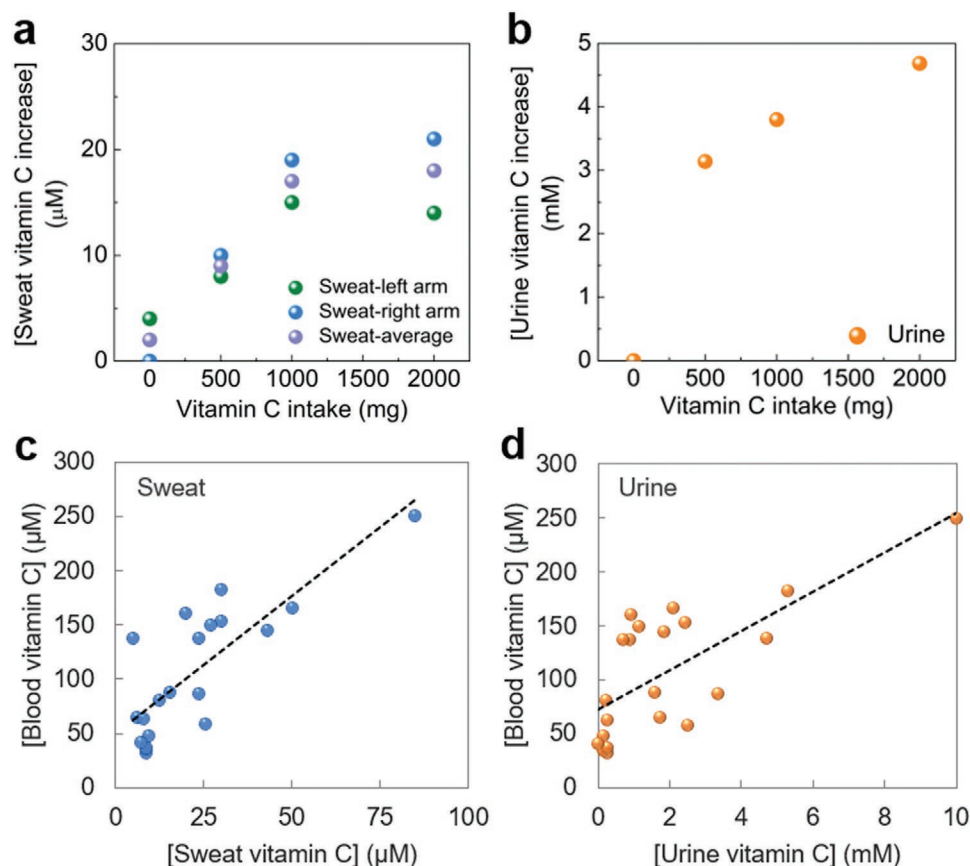


Figure 5. Monitoring sweat, urine, and blood with different vitamin C intake on human subjects. a) Relationship between the sweat-vitamin C concentration and vitamin C intake. b) Relationship between the urine-vitamin C concentration and vitamin C intake. Results in (a) and (b) are based on a single subject. c) Relationship between the sweat and blood vitamin C concentration. ($r = 0.8071$, $p = 0.00002$). d) Relationship between urine and blood vitamin C concentration. ($r = 0.72$, $p = 0.0002$). Results in (c) and (d) are based on 21 out of 21 tests on two subjects.

conducted by applying a pulsed voltage from 0 to -2 V with 50% duty cycle at 50 Hz. PEDOT:LiClO₄ deposition was realized in 1× PBS (pH 7.4) solution containing 0.02 M EDOT and 0.02 M LiClO₄ at a polymerization potential of 1.1 V versus Ag/AgCl for 60 s. After deposition, 5 μ L of 2.5 mg mL⁻¹ ascorbate oxidase was drop-casted onto the electrode surface and left in room temperature for 5 h for drying. Finally, 4 μ L of 0.5% Nafion solution was covered on the electrode surface, and the electrode was left to dry overnight under ambient environment. The reference electrode was prepared by dropcasting a thin layer of silver ink and cured at 80 °C.

Sensor Characterization: Morphologies of the vitamin C sensors were characterized using field-emission SEM (JSM-7100F, Japan). Cyclic voltammetry and amperometric response ($i-t$) of the sensors corresponding to different concentrations of vitamin C were evaluated using an electrochemical workstation (CHI 1220B, USA). The temperature influence experiments were performed using the same sensor in four Petri dishes containing solutions at different temperatures on different hot plates. Furthermore, pH influence experiments were carried out using the same sensor in four Petri dishes containing solutions with different pH. All measurements were paused while refreshing solutions and continued after a waiting period of 2 min.

Iontophoresis Sweat Analysis: The human subject trials were conducted in accordance with the protocol (CPHS 2015-05-7578) approved by the Institutional Review Board at the University of California, Berkeley and following informed written consent. Six healthy male subjects, aged 20–30, were recruited from the University of California, Berkeley campus. Subjects had vitamin C (Emergen-C) for the trials. Measurements of the vitamin C concentrations in biofluids were done using an electrochemical work station (CHI 1220B, USA). Prior to iontophoresis, subjects' wrists were cleaned with an alcohol swab and gauze. The iontophoresis was conducted using ELITechGroup Model 3700 Webster Sweat Inducer. Subsequently, sweat was collected with commercial Macroduct sweat collectors for \approx 30 min. Finally, the samples were evaluated using the prepared vitamin sensors. Before and after each test, sensors were tested in standard PBS solution with subsequent additions of vitamin C. For the trials indicated in Figure 3, the first sweat and urine samples were collected from the subjects at 9:00–9:30 am. Subjects consumed 1000 mg vitamin C tablets at 10:00 am. 3 h later (1:00–1:30 pm), second sweat and urine samples were collected. At 6:00 pm, the last sweat and urine samples were collected. The studies in Figure 4 were conducted in two consecutive days. To reduce external interference, the subjects minimized consumption of vitamin C rich food during the period of experiments and drank water (\approx 500 mL) every 3 h for the trials with controlled water intake. For sweat tests, sensors were installed on the wrist where there would be minimal to no bending. For blood tests, a drop of blood was directly dropped on top of the sensor for immediate measurement. The sensors were used for one time in each test.

Statistical Analysis: All data are presented without further processing unless specifically stated. The data were plotted through the Origin. The standard deviations (as indicated by using the STDEV function on Microsoft Excel) and the sample sizes are displayed in the relevant figures. r and p values were computed from Matlab with corrcoef function for a two-tailed test. There was no removal or adjustment of the collected data.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

J.Z. and H.Y.Y.N. contributed equally to this work. A.J., J.Z., and H.Y.Y.N. conceived the idea and designed the experiments. J.Z. and H.Y.Y.N. performed tests and analysis on sensors and human studies. L.H. assisted in human studies and sensor characterization. Y.L., M.B., and C.H.A. helped with sensors' tests. W.J. assisted with device fabrication.

Keywords

flexible sensors, nutritional assessment, sweat sensors, wearable electronics

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