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Article

Epidermal Enzymatic Biosensors for Sweat Vitamin C: Toward Personalized Nutrition

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Cite This: *ACS Sens.* 2020, 5, 1804−1813

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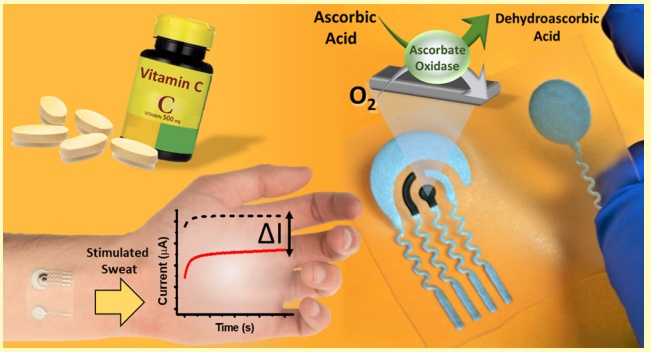
ABSTRACT: Recent advances in wearable sensor technologies offer new opportunities for improving dietary adherence. However, despite their tremendous promise, the potential of wearable chemical sensors for guiding personalized nutrition solutions has not been reported. Herein, we present an epidermal biosensor aimed at following the dynamics of sweat vitamin C after the intake of vitamin C pills and fruit juices. Such skin-worn noninvasive electrochemical detection of sweat vitamin C has been realized by immobilizing the enzyme ascorbate oxidase (AAOx) on flexible printable tattoo electrodes and monitoring changes in the vitamin C level through changes in the reduction current of the oxygen cosubstrate. The flexible vitamin C tattoo patch was fabricated on a polyurethane substrate and combined with a localized iontophoretic sweat stimulation system along with amperometric cathodic detection of the oxygen depletion during the enzymatic reaction. The enzyme biosensor offers a highly selective response compared to the common direct (nonenzymatic) voltammetric measurements, with no effect on electroactive interfering species such as uric acid or acetaminophen. Temporal vitamin C profiles in sweat are demonstrated using different subjects taking varying amounts of commercial vitamin C pills or vitamin C-rich beverages. The dynamic rise and fall of such vitamin C sweat levels is thus demonstrated with no interference from other sweat constituents. Differences in such dynamics among the individual subjects indicate the potential of the epidermal biosensor for personalized nutrition solutions. The flexible tattoo patch displayed mechanical resiliency to multiple stretching and bending deformations. In addition, the AAOx biosensor is shown to be useful as a disposable strip for the rapid in vitro detection of vitamin C in untreated raw saliva and tears following pill or juice intake. These results demonstrate the potential of wearable chemical sensors for noninvasive nutrition status assessments and tracking of nutrient uptake toward detecting and correcting nutritional deficiencies, assessing adherence to vitamin intake, and supporting dietary behavior change.



KEYWORDS: *ascorbate oxidase, vitamin C sensor, wearable biosensor, sweat ascorbic acid, iontophoresis*

Wearable chemical sensors have recently received tremendous attention toward the monitoring of the wearer's health and fitness.[1,2] Newly developed wearable bioelectronic devices have thus been shown useful for tracking continuously and noninvasively (bio)chemical markers, such as metabolites and electrolytes, in different body fluids.[3] With the tremendous progress in wearable sensor technology, there are growing interest in exploring new applications and market opportunities, beyond the management of diseases or performance assessment. For example, wearable sensors have been proposed recently for monitoring drug abuse or assessing medication compliance,[4−7] and new wearable devices have been reported for detecting alcohol[8−10] and opioid abuse.[11] On the other hand, the use of wearable biosensors for precision nutrition has rarely been explored. While a variety of wearable physical sensors (piezoelectric, acoustic, and EGG) have recently been proposed for monitoring food intake via swallowing and chewing,[12] there are no reports on developing wearable chemical sensors for monitoring eating habits.

The ability to monitor continuously and noninvasively the intake of food and vitamins could greatly support dietary and nutrition behavior change.[13,14] The use of wearable sensors for noninvasive nutrient monitoring can thus be extremely useful for preventing and managing nutritional imbalance, and for

Received: March 25, 2020
Accepted: May 5, 2020
Published: May 5, 2020

https://dx.doi.org/10.1021/acssensors.0c00604
ACS Sens. 2020, 5, 1804−1813

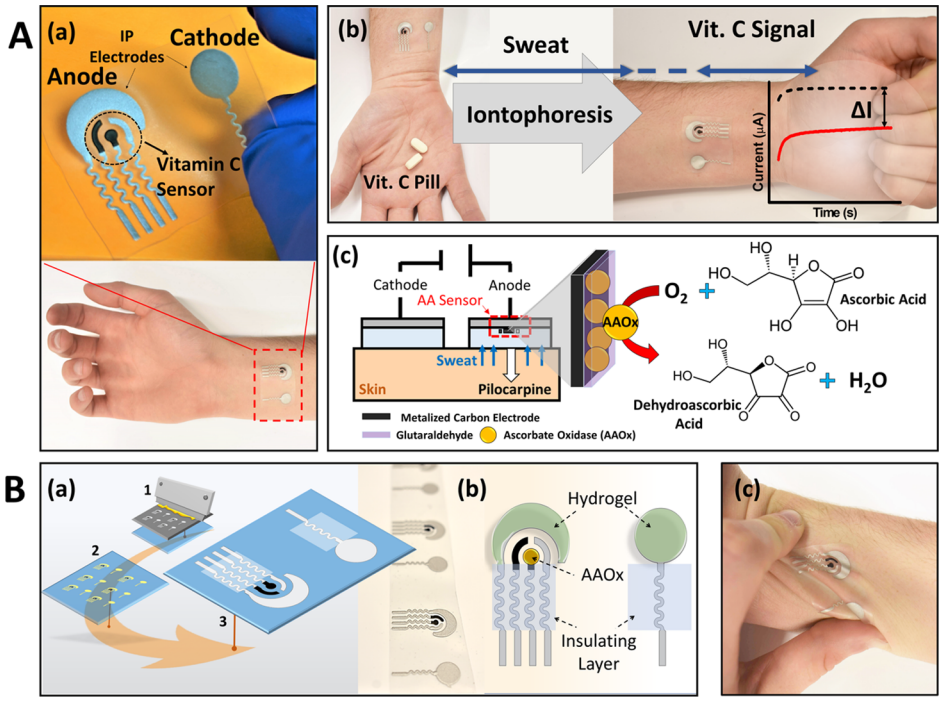


Figure 1. Ascorbic acid (vitamin C) determination in stimulated sweat. (A) (a) Electrode design for simultaneous sweat stimulation and detection. Sweat is stimulated by IP delivery of pilocarpine (located in the anode compartment) using the cathode and anode electrodes. Amperometric vitamin C detection is performed by using a three-electrode system located on the anode compartment. (b) Protocol used for the biosensing of ascorbic acid. Sweat is stimulated before (black dotted line) and after (red solid line) taking vitamin C pills; the vitamin C response is based on the difference in the current before and after taking the pill. (c) Schematic of the localized sweat stimulation using IP pilocarpine delivery and of the enzymatic reaction for detecting ascorbic acid on a metalized Rh-carbon printed electrode. Pilocarpine is delivered on the anode, where the AAOx-immobilized sensor using glutaraldehyde is located; the amount of oxygen consumed by the enzymatic reaction and hence the vitamin concentration is measured from the reduction current of oxygen. (B) (a) Fabrication of the vitamin C biosensor. 1- Screen printing using Ag/AgCl for the IP electrodes, reference and current collectors, and Rh-carbon ink for the counter and working electrodes. 2—printed and cured electrodes. 3—printing an insulating layer to define the electrode area. (b) Schematic showing the location of the hydrogel and the enzyme layer. On the anode, agarose (loaded with 2% pilocarpine) was used, and on the cathode, agarose in 0.1 M PBS was used. (c) Image of the epidermal sensor under mechanical (twisting) strain.

providing personalized guidance toward maintaining desirable nutrient levels. These could be particularly important for maintaining balanced vitamin levels in the body where controlled supplementation and frequent vitamin measurements are urgently needed.[15−17] However, conventional vitamin monitoring techniques are usually time consuming, rely on the use of expensive and specialized instruments, or require invasive blood sampling .[18] In addition, and specific for vitamin C, the biological fluids need to be stabilized, right after sampling, and maintained at low pH (e.g. concentrated metaphosporic acid) and low temperature until analyzed in the lab.

Herein, we describe a disposable, noninvasive, flexible and printable wearable electrochemical sensor for epidermal monitoring of the intake, and dynamics, of vitamin C in sweat and demonstrate the potential of such device toward personal nutrition and wellness. Vitamin C, also known as L-ascorbic acid (AA), is a water-soluble nutrient vital for the normal growth and physiological function of the human body.[19] This vitamin plays a major role in a number of bodily functions including the production of collagen (and related wound healing and skin care), in the absorption of iron, treatment of cold, and in helping the immune system to protect the body against viral infections and neurological disorders, as well as in cardiac, gum, and even cancer diseases.[20−26]

The determination of vitamin C in biological fluids is thus of considerable importance toward preventing vitamin imbalance and minimizing the risks of such disorders and diseases. However, studies of vitamin C in sweat or saliva are scarce, and related on-body monitoring has not been demonstrated so far.[27] Various analytical methods have been used to quantify the concentration of vitamin C.[20,26] However, most of these techniques rely on costly and bulky instruments [e.g., high-performance liquid chromatography (HPLC)] that are not suitable for decentralized nutrition assessment. Voltammetric measurements at bare or nanomaterial-modified electrodes, while suitable for on-the-spot testing, suffer from limited specificity, because of the presence of co-existing electroactive constituents.[20,29]

The new wearable vitamin C bioelectronic sensor relies on the immobilization of ascorbate oxidase on screen-printed electrodes for amperometric determination of vitamin C in stimulated sweat (Figure 1A). In this epidermal biosensor, the immobilized ascorbate oxidase enzyme catalyzes the oxidation of vitamin C to dehydroascorbic acid by consuming oxygen. The amount of oxygen consumed by the enzymatic reaction is proportional to the ascorbic acid concentration, and it can be measured by monitoring the changes in the oxygen reduction current.[30,31] The flexible vitamin C bioelectronic patch, fabricated on a polyurethane substrate, combines the AAOx-enzymatic biosensing with a pilocarpine-based iontophoretic system for direct sweat stimulation (Figure 1B). Deformation tests performed by stretching and bending the iontophoretic (IP)−amperometric tattoo patch demonstrated great resiliency against such severe strains. To the best of our knowledge, this represents the first

https://dx.doi.org/10.1021/acssensors.0c00604
ACS Sens. 2020, 5, 1804−1813

enzymatic electrochemical sensor for the epidermal detection of sweat vitamin C, and the first example of a noninvasive sensing device capable of tracking dynamic trends of vitamins following the intake of supplements. We were able to follow noninvasively and qualitatively the temporal profile of vitamin C physiology in sweat, after taking one vitamin C pill, after taking several vitamin C pills, and after taking a commercial vitamin C-rich beverage. In addition, we demonstrate the possibility of using the AAOx biosensor for rapid on-the spot in vitro measurements of vitamin C in collected saliva and tears. The new epidermal platforms can be adapted for the noninvasive monitoring of other essential vitamins for empowering people to take greater control of their diet toward precision nutrition and vitamin compliance.

EXPERIMENTAL SECTION

Chemicals and Instruments. Ascorbate oxidase (from cucurbita, 10−40 units mg^{-1} protein), rhodium-on-carbon, glutaraldehyde solution (50%), agarose type IV, pilocarpine nitrate, silver flacks (10 μm size), low molecular weight chitosan, high molecular weight poly(vinyl chloride), bovine serum albumin, potassium phosphate monobasic (K_2PO_4), potassium phosphate dibasic (K_2HPO_4), ethanol, acetone, hydrochloric acid, L(+)-ascorbic acid, uric acid, caffeine, acetaminophen polystyrene-*block*-polyisoprene-*block*-polystyrene (SIS, D1117 PT) polymer balls, and toluene were obtained from Sigma-Aldrich (St. Louis, MO). Carbon paste ink was received from Gwent. A thermoplastic polyurethane film was obtained from American Polyfilm Inc. Silver conductive epoxy adhesive 8331-14G was obtained from MG Chemicals. All reagents were used without further purification. Vitamin C pills (1000 mg) were received from Nature Made nutritional products. Menthol tear stick (Art. #3005) was obtained from Kryolan, CA. Electrochemical measurements were performed at room temperature using an EmStat3 (PalmSens) and an Autolab PGSTAT101 potentiostat/galvanostat controlled using PSTrace v 5.4 and NOVA v 1.11.2 software, respectively.

Sensor Fabrication. Screen printing technique was used to fabricate the ascorbic acid biosensor, as shown in Figure 1B(a). First, the desired electrode pattern was designed with AutoCAD software (Autodesk, San Rafael, CA) and fabricated on stainless-steel, using a 12 in. × 12 in. hole, framed stencil (Metal Etch Services, San Marcos, CA). The electrode array consisted of two IP electrodes for sweat stimulation (cathode and anode), along with a Ag/AgCl reference electrode, a carbon counter electrode, and a sensing working electrode fabricated with a metalized (1 wt% rhodium) carbon ink (Gwent). The current collectors were printed using stretchable silver ink, which is fabricated as follows. The stretchable Ag/AgCl silver ink was prepared by mixing three parts of silver flakes with two parts of SIS binder. SIS binder was prepared by mixing 4 g of SIS in 8.76 g of toluene until a clear solution was formed (using a vortex shaker). The device fabrication was realized in several steps. First, the serpentine current collectors were printed on a flexible polyurethane substrate using the stretchable silver ink, followed by printing a pair of IP electrodes and then the reference electrode using the Ag/AgCl ink. The counter and working electrodes were subsequently printed using the metalized (1 wt% Rh) carbon modified Gwent graphite ink. After each step, the printed ink pattern was cured at 50 °C for 10 min in a conventional oven. Finally, an insulating Ecoflex layer was printed to define the electrode areas.

Sensor Modification. Several metalized carbon inks were tested for enhanced ascorbic acid detection (Figure S1). The optimized 1 wt% Rh carbon working electrode showed the best performance and was functionalized with the vitamin C recognition layer accordingly to the following protocol. A volume of 2 μL of AAOx enzyme (0.1 IU) mixture containing 100 IU of a commercial 1:9 AAOx/BSA (10 mg/mL) in 0.1 M phosphate-buffered saline (PBS) stabilizer was applied to the working electrode by drop-casting. Next, 0.5 μL of glutaraldehyde (0.5%) was cast on the AAOx-modified electrode (optimization of enzyme modification is shown in Figure S3B). Then, the enzyme-immobilized electrode was placed in the fridge at 4 °C overnight.

Electrochemical Detection. Electrochemical detection of ascorbic acid was commonly carried out using chronoamperometry (CA), and in a few cases using square wave voltammetry (SWV). Optimization of the SWV and CA parameters is shown in Figures S2 and S3A. A PalmSens4 potentiostat was used for both techniques with the following parameters. SWV was performed from −0.4 to +1.0 V, with a step potential of 0.004, an amplitude of 0.05 V, and a frequency of 20 Hz. A constant potential of −0.5 V for CA was used.

IP Gels. For the IP process, agarose hydrogel was used at the cathode compartment, and agarose gel loaded with pilocarpine was used at the anode compartment. The cathode agarose hydrogel was prepared by heating at 170 °C a continuously stirred agarose solution (4% w/v) with 0.1 M potassium phosphate buffer (pH 7.0) until it completely dissolved. Similarly, the anode pilocarpine-loaded hydrogel was prepared by heating at 170 °C a continuously stirred agarose solution (4% w/v) with water until complete dissolution. Subsequently, the heat was switched off and 2% pilocarpine nitrate powder was added with continuous stirring. Note that mixing pilocarpine with agarose powder under heat of 170 °C will lead to pilocarpine decomposition, hence sweat will not be generated. Thin layered discs of the as-prepared gels were fabricated by casting the gel in a flexible silicon disc mold (2 cm diameter, 3 mm thickness, and the anodic gel was cut to the same dimensions as the cathode IP electrode (Figure 1B(b)). The as-prepared thin layers of gel were stored in the fridge in a wet chamber.

On-Body Measurements. Epidermal evaluation of the device was performed on healthy consenting subjects with no prior history of heart conditions, diabetes, or chronical pain, and in strict compliance with the protocol approved by the Institutional review board (IRB) at the University of California, San Diego. The device was placed in the forearm of the volunteers for all on-body evaluations. For all cases, sweat was stimulated by the FDA-approved iontophoretic delivery of pilocarpine with no harm or pain to the volunteer. For this, sweat was stimulated by using a μAutolab III electrochemical analyzer to apply a current density of 0.3 mA cm^{-2} to the cathode and anode electrodes for 10 min. A single prestep of skin conditioning was performed by applying the same current density using agarose gels in the cathode and anode compartments for 10 min, followed by immediate placement of the device with pilocarpine delivery gels on the conditioned area. Before the placement of the sensor, the skin was thoroughly cleaned with soap and rubbing alcohol. The vitamin C patch was transferred to the skin by using a double-sided clean laser tattoo transfer adhesive (Papilio, TM). Openings were made in the adhesive film to expose the vitamin C sensor and IP electrodes (with IP gels) to the skin (Figure 5A). A single device was used for each volunteer for following the transient sweat vitamin C response, while repeating the 10 min iontophoresis up to 4 times. The electrodes were connected to the μAutolab III and EmStat3 (PalmSens) potentiostats during the IP and detection steps, respectively.

Sweat Stimulation. The optimum IP duration time for sweat stimulation (toward subsequent ascorbic acid determination) was determined by applying several stimulation times of 5, 10, and 15 min. Because of the preconditioning step (10 min using agarose gel in both the cathode and the anode), no significant difference was found in the vitamin C signal for different IP times (Figure S4), and a 10 min stimulation was used to ensure efficient pilocarpine delivery, regardless of the skin type.

Vitamin C Pill Intake. The volunteers were recruited after obtaining their informed written consent for on-body testing and evaluation of the epidermal biosensor before and after taking vitamin C pills. In all the experiments, the vitamin C epidermal biosensor was placed on the subject's forearm, and the sweat measurements were conducted by applying IP followed by chronoamperometric operations to record the current response of sweat vitamin C concentration before and after taking vitamin C pills. During the IP sweat stimulation, a constant current density of 0.3 mA cm^{-2} was applied for 10 min between the iontophoretic anode and cathode electrodes to deliver pilocarpine to the skin through the agarose hydrogel layer and generate sweat. The applied current density was chosen based on previous studies.[8,32] After sweat stimulation, a resting time of 2 min was allowed for sweat generation. This was followed by amperometric measure-

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ACS Sens. 2020, 5, 1804−1813

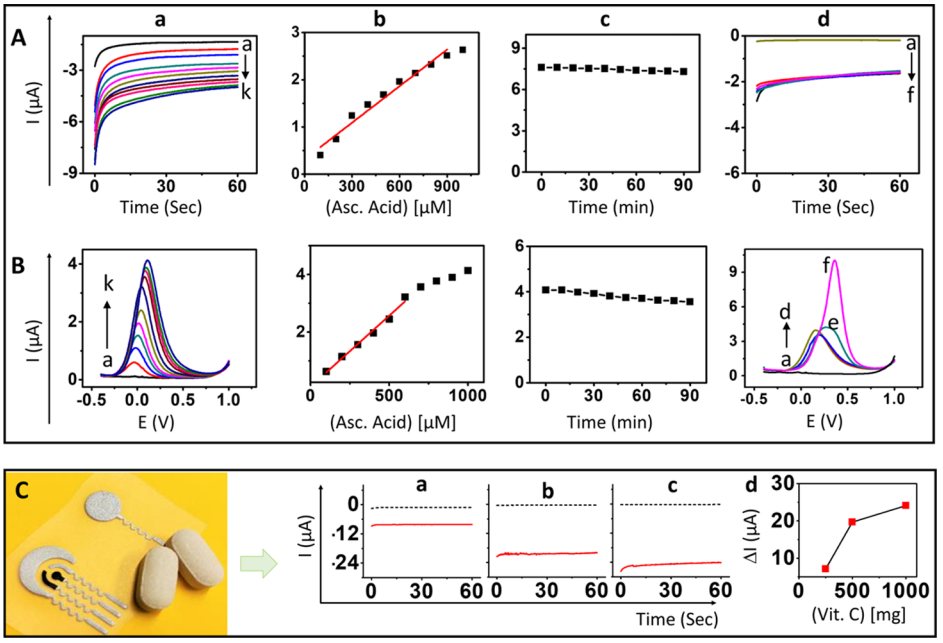

Figure 2. In vitro and on-body characterization of the epidermal vitamin C sensor: a comparison of the amperometric-enzymatic (A) and nonenzymatic-SWV (B) sensors. (A) (a) Chronoamperometric response of ascorbic acid in PBS over the 100−1000 μM concentration range (a−k), along with the corresponding calibration plot (b). (c) Stability of the 1 mM vitamin C response recorded at 10 min intervals. (d) Selectivity testing in buffer solution: a- PBS baseline, b- vitamin C, c- caffeine, d- glucose, e- uric acid, and f- acetaminophen. (B) (a) SWV response to increasing ascorbic acid concentrations in PBS from 100 to 1000 μM (a−k), along with the corresponding calibration plot (b). (c) Stability testing for the 1 mM vitamin C signal measured every 10 min and (d) selectivity testing response in buffer solution: a- PBS baseline, b- vitamin C, c- caffeine, d- glucose, e- uric acid, and f- acetaminophen. (C) Response of the enzymatic biosensor to variable-sized vitamin C doses. Amperometric response of the sweat sensor recorded 90 min after taking (a) a half pill containing 250 mg, (b) one pill with 500 mg, and (c) two 500 mg pills with a total of 1000 mg of vitamin C along with (d) the corresponding plot of current vs amount of vitamin C consumed.

ments of the sweat ascorbic acid concentration at an applied potential of −0.50 V (vs Ag/AgCl). Such iontophoretic/detection operation was carried out before taking vitamin C pills or juice, and at given time intervals after the intake of pills or juice. The current response before vitamin C intake served as a baseline, corresponding to the physiological sweat vitamin C concentrations. The increasing in the level of vitamin C, because of the supplement intake, was estimated by measuring the difference between the currents before and after taking the pill. The former is significantly smaller than the response recorded after taking the pill.

The amount of vitamin C in sweat due to the supplement or drink intake was measured before and after taking different amounts of pill supplements or fruit juices. For the time-dependent temporal profile of vitamin C in sweat, the subjects were asked to consume one commercial vitamin C pill (1000 mg), and the amperometric signal was obtained every 45 min until it reached the baseline. For different amounts of vitamin C in a fixed time, the subjects were asked to take a 250 mg vitamin C pill and the signal was measured after 45 min, followed by the ingestion of a second 250 mg vitamin pill, and another 45 min later, after taking the second pill, the signal was again recorded. Next, a third 250 mg pill was taken, followed by recording the corresponding signal after 45 min.

Several control experiments were carried out to ensure that the sensor response was specific to vitamin C in the sweat. The first control experiment was performed without taking any pills while recording the amperometric response every 45 min for 3 h. The second control experiment relied on an enzyme-free electrode along with a 1000 mg pill intake and measurement every 45 min for 3 h. Both control experiments were performed under identical conditions as the actual experiments.

Juice Intake. Different volumes of orange juice (160, 320, and 640 mL) containing 90 mg of vitamin C per 240 mL were used in the study. Different volumes, corresponding to a 160 mL glass, were taken, and after 90 min, sweat was stimulated, and the vitamin C amperometric determination was performed in a similar fashion as the multiple pill experiment.

Tears and Saliva. Tear samples (70 μL) were collected from a volunteer every 45 min over 3 h using an Eppendorf. Tear stimulation was performed by using a menthol tear stick. The menthol stick was applied at ∼1 cm under the volunteer's eye, as per previous study.[33] Electrochemical vitamin C determination from the collected tear sample (70 μL) was carried outside of the body without any treatment of the sample.

Saliva samples from a subject were collected every 15 min until 1 h and after 2 h by using the already reported "passive drool method".[34] The collected raw saliva samples were directly employed for the electrochemical measurements without any treatment. In brief, 70 μL of the collected saliva was placed on the electrode surface, and electrochemical measurements were conducted by applying a constant potential (−0.50 V) for 60 s. Control experiments for saliva vitamin C were conducted in a similar fashion as for sweat vitamin C.

Mechanical Deformation. The mechanical integrity and skin conformability of the vitamin C tattoo biosensor were examined through repetitive mechanical strains involving bending and twisting. For the skin conformability, the flexible screen-printed device was transferred to the volunteer forearm, and the device was twisted on the skin 50 times. For testing the mechanical resiliency, the device was bent inward and outward 50 times followed by 50 stretchings (10%). After each cycle of mechanical deformation, the amperometric response for 500 μL vitamin C was measured in vitro (in comparison with the signal of the pristine sample).

RESULTS AND DISCUSSION

The IP electrodes were integrated with an electrochemical sensing system on the same flexible tattoo platform (Figure 1A). The device geometry of each electrode (sensor and IP electrodes) was carefully designed to ensure successful localized sweat stimulation and selective vitamin C amperometric detection. The anode and the cathode were placed 2 cm apart

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ACS Sens. 2020, 5, 1804−1813

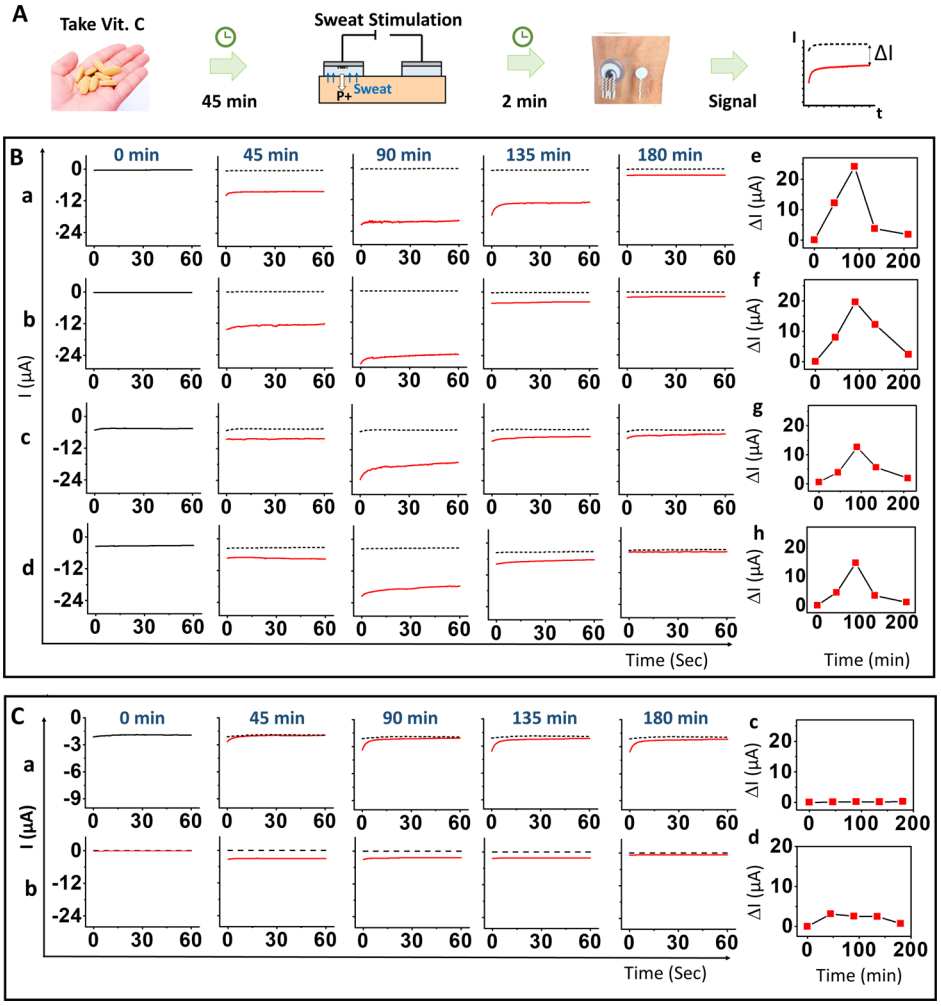

Figure 3. On-body test for vitamin C detection in stimulated sweat. (A) Schematic and timeline for the sweat stimulation (using IP delivery of pilocarpine) and detection process. The sweat vitamin C response was obtained before taking the vitamin (dotted black line) and every 45 min after taking a 1000 mg vitamin C tablet (red solid line). Sweat vitamin C was recorded after 2 min of sweat stimulation. (B) Typical profile for vitamin C in sweat obtained for four subjects. Amperometric response of ascorbic acid in stimulated sweat after taking a 1000 mg pill and recording the signal at 45 min intervals for four subjects (a–d); the corresponding sweat vitamin C temporal profiles of individual subjects (e–h). (C) Control response (a) without taking the vitamin pill and (b) without enzyme modification (but with pill intake), followed by measurements at 45 min intervals.

from each other to ensure efficient IP-based sweat extraction.[8,32,35] The IP hydrogel was prepared in a PBS solution to protect the epidermis from pH changes during repeated stimulations and sensing as a result of ionic build-up at the sweat sampling site. Pilocarpine was used for sweat stimulation. As pilocarpine is positively charged, it was placed on the anode compartment where a positive current was applied for its delivery via electrostatic repulsion (Figure 1A(c)). The vitamin C biosensor was located as close as possible to the anode, where the sweat was stimulated. The anodic pilocarpine-containing IP gel did not contact the enzyme biosensor, hence preventing pilocarpine interference in the amperometric measurements and sweat intake by the iontophoretic gel (Figure 1B(b)). The cathodic IP electrode was designed for ensuring comfort during IP (with no apparent burning sensation) and had a similar area as the anode.

Stimulated sweat from the immediate vicinity of the anode was used for detecting vitamin C (Figure S5). Ascorbate oxidase was immobilized on the Rh-metalized carbon electrode via glutaraldehyde crosslinking, with the metalized carbon transducer offering enhanced electrocatalytic detection of the depleted oxygen. The reactions involved in such vitamin C detection are shown in Figure 1A(c). The enzyme ascorbate oxidase catalyzes the oxidation of ascorbic acid to dehydroascorbic acid by consuming oxygen. Hence, quantification of the sweat vitamin C concentration is performed by correlating the decreased oxygen–reduction response upon increasing the vitamin concentration.[20] The oxygen depletion thus increases upon increasing the vitamin C concentration. The amount of oxygen consumed by the enzymatic reaction can be measured by the reduction current of oxygen. The main reactions, enzymatic (reaction 1) and electrochemical detection (reaction 2), in such biosensing of ascorbic acid in the presence of immobilized AAOx are given by [31,36,37]

$$\text{L-ascorbic acid} + 1/2O_2 \xrightarrow{\text{AAOx}} \text{dehydroascorbic acid} + H_2O \quad (1)$$

$$O_2 + 4e^- + 2H^+ \rightarrow H_2O \quad (2)$$

The electrode design eliminates the need for an independent protocol for sweat stimulation, sampling, and analyte determination by combining all these features in a single platform.

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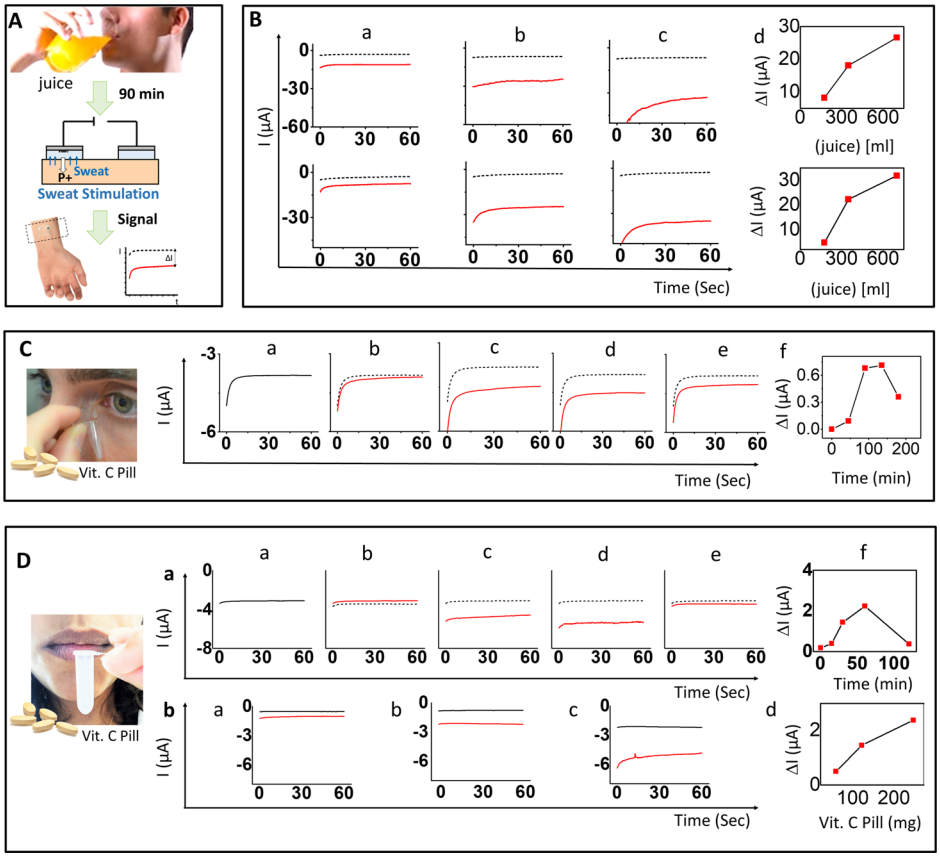

Figure 4. Vitamin C enzymatic sweat detection in mixed fruit juice, tears, and saliva. (A) Schematic and (B) response of the epidermal biosensor 90 min after drinking a- half, b- one, and c- two cups of mixed fruit juice (containing pineapple, banana, and orange; in total, 0.4 mg vitamin C/mL), along with the (d) plot of the corresponding current response vs volume of juice for two subjects (top and bottom). (C) Amperometric response of a disposable AAOx-biosensor strip to ascorbic acid in tears before (a) and at 20 min intervals after (b−e) taking a 1000 mg vitamin C pill and the corresponding plot of current vs time (f). (D) (a) Amperometric response of a disposable AAOx-biosensor strip to ascorbic acid in saliva (a) before and (b−e) after taking 1000 mg vitamin C pill at 20 min intervals; (f) the corresponding plot of current vs time. (D) (b) Response after 60 min of taking 62.5 (a), 125 (b), and 250 mg of vitamin C pill (c). (d) The respective signal response vs amount of vitamin C. Applied potential, −0.5 V.

The analytical performance of the enzymatic amperometric vitamin C biosensor was compared with that of the SWV-based nonenzymatic electrode; for this, metalized (Rh 1 wt%) carbon electrodes were used in both cases. CA and SWV were thus used for the AAOx enzyme-modified, and nonenzymatic sensors, respectively. Such comparison indicates that the enzymatic sensor displays a more favorable analytical performance, particularly, a higher selectivity and stability and a wider dynamic range, along with good reproducibility (RSD 3.9%, Figure S6). Figure 2A(a),B(a) displays the response of these CA and SWV sensors to PBS buffer solutions containing increasing vitamin C levels over the 0−1000 μM range. While the amperometric enzymatic sensor displays good linearity over the entire range, the non-enzymatic voltammetric sensor yields a linear response up to around 600 μM, with a slight curvature at higher levels. The well-defined response to such micromolar vitamin C concentrations indicates the suitability of the device for practical on-body testing (described below), in view of the expected micromolar levels of vitamin C in sweat.[27,28] The AAOx biosensor also displays a slightly higher stability during a prolonged 90 min operation, as shown in Figure 2A(c),B(c). Such operational stability meets the demands of this study, that is, tracking the rise and fall of vitamin concentrations occurring within the first 2 h after the vitamin C intake, as illustrated in Figure 3. The corresponding sensor stability CA and SWV voltammograms are displayed in Figure S7A(a),B(a), respectively. Furthermore, the enzyme-modified sensor offered a highly selective response in the presence of several relevant electroactive sweat constituents, such as uric acid, acetaminophen, caffeine, and glucose (Figures 2A(d) and S7Ab). In contrast, the nonenzymatic SWV sensor displays significant interferences (overlapping, distorted, and larger peaks) from uric acid (e) and acetaminophen (f) that reflect their anodic response (Figure 2B(d)).

Following the in vitro evaluation, the skin-worn AAOx biosensor was evaluated for its ability to detect vitamin C in sweat. The sensor performance was examined firstly by taking multiple vitamin pills and monitoring the sweat response after 90 min (Figure 2C). An increasing current response for vitamin C in the stimulated sweat is clearly observed following the intake of 250, 500, and 1000 mg of vitamin C (Figure 2C), indicating that the sensor responds favorably to variations in vitamin C sweat levels. Note, however, that the response for 500 and 1000 mg of vitamin C are nearly similar, reflecting the body's limitation in absorbing large amounts of vitamin C.[38] It is also important to notice that direct translation of the current response values to concentrations (through in vitro precalibration of the corresponding electrode batch, similar to those of Figure 2A) will facilitate the desired quantitation. Such quantitation is beyond the scope of this proof-of-concept study which aims at introducing a noninvasive sensor for

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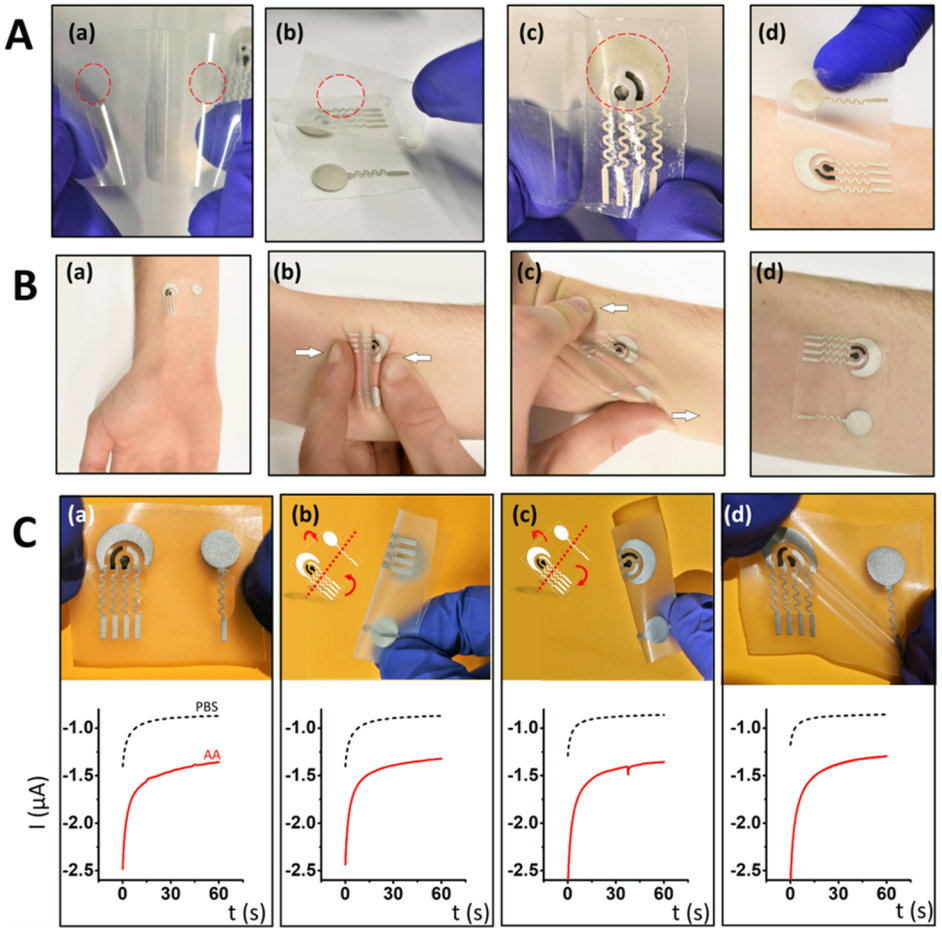

Figure 5. Mechanical resiliency of the epidermal vitamin C biosensor. (A) (a) Removing the first protective layer from the double-sided tattoo adhesive with an opening for the sensing area (b). Applying adhesive to the tattoo (c). Removing the second protective layer from the applied adhesive and (d) transferring to the body. (B) (a) Skin conformability and mechanical integrity of the vitamin C biosensor, including (b) bending, (c) twisting, and (d) the biosensor condition after twisting and bending. (C) Visual (top) and electrochemical (bottom) mechanical integrity of the vitamin C biosensor (a) before stretching and bending, (b) after a 180° inward bend, (c) after a 180° outward bend, and (d) after stretching. All the deformations were repeated 50 times on a single device. The electrochemical tests were performed in vitro after each set of deformation in PBS 0.1 M and by spiking 500 μM of vitamin C.

tracking qualitatively the dynamics of vitamin C following food or supplement intake.

Based on the attractive analytical performance of the epidermal tattoo biosensor, we evaluated the temporal profile of sweat vitamin C by measuring the response in several human healthy subjects every 45 min after the intake of a single 1000 mg of vitamin C pill (Figure 3). Sweat was stimulated for 10 min, and the vitamin C sensor response was measured within 2 min, as shown in Figure 3A. The change in the sweat vitamin C signal (ΔI) was estimated by measuring the difference between the signal after taking the vitamin pill (red solid line) and the initial baseline recorded before taking the vitamin (black dotted line). Such current difference is proportional to the change in the amount of vitamin C in the generated sweat because of the pill intake (with the initial current reflecting the concentration without the pill). The results of the amperometric response of sweat vitamin C of four volunteers at 0, 45, 90, 135, and 180 min after taking the 1000 mg pill are shown in Figure 3B(a−d). The maximum vitamin C signal (ΔI) was achieved 90 min after taking the vitamin supplement, followed by a decreasing signal, approaching the baseline, after 180 min[38] The corresponding sweat vitamin C temporal profiles, shown in Figure 3B(e−h), reflect the pharmacokinetic profile of vitamin C—including the changes in plasma vitamin C—as described in previous studies.[38,39] Such different profiles of different subjects to a given amount of vitamin C—with different maximum currents and specific current changes—indicate the potential of the epidermal biosensor toward personalized nutrition applications. In order to further assess the sensor performance, control evaluations were performed by conducting the same experiments without taking any vitamin pill. As indicated by Figure 3C(a), only a negligible current signal was observed without the pill intake, indicating that the sensor response is solely due to the presence of vitamin C, with no contributions from other sweat constituents. This control study also demonstrates that no oxygen fluctuations occur throughout the experiment. An additional control experiment, without the immobilized AAOx enzyme but with the intake of a 1000 mg vitamin C pill, was also performed. As expected from the absence of the enzymatic reaction, no apparent current response is observed (Figure 3C(b)), reinforcing the crucial role of AAOx in generating the sweat vitamin C response.

After the successful monitoring of vitamin C in sweat, the biosensor was evaluated using different relevant dietary scenarios. In most common situations, the main source of vitamin C comes from the intake of fruits and vegetables in the

https://dx.doi.org/10.1021/acssensors.0c00604
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diet. Therefore, to monitor such vitamin intake from food, the wearable sensor patch should be able to measure vitamin C concentrations in our body after food intake. To demonstrate this capability, on-body sensing experiments were carried out to monitor the changes in vitamin C sweat levels after the consumption of different quantities of juice. A mixed fruit juice containing pineapple, banana, and orange, with a vitamin C concentration of 0.4 mg/mL, was selected as the source of vitamin C. Two human subjects were asked to drink 0.5, 1, and 2 cups of juice on separate sessions, and their vitamin C levels in stimulated sweat were measured 90 min after the intake of juice (Figure 4A). As illustrated in Figure 4B, the epidermal sensor displayed higher current signals for both subjects, reflecting the increased vitamin C sweat levels, following the intake of gradually larger volumes of juice. The difference in the current response of the two individuals reflects the differences in their weights, metabolic rates, and other factors, which will be a topic of a separate detailed future study focusing on personalized nutrition.

Because of its complex metabolism and pharmacokinetics, the concentration of vitamin C can vary with the type of biofluid and time. Accordingly, we evaluated the ability of the enzymatic sensor to measure vitamin C concentrations in different biofluids. Two easily accessible biofluids, tears and saliva, were chosen for this demonstration in connection to the disposable AAOx-strip biosensor. It is important to notice that the optimal pH range of the AAOx enzyme, pH 5.5−7.0, is close to the different pHs of the biofluids tested in this study (sweat ≈ pH 5, tears ≈ pH 7, and saliva ≈ pH 7.3). Tear vitamin C was evaluated every 20 min intervals after taking 1000 mg of vitamin C pill. The results shown in Figure 4C indicate a similar vitamin C temporal profile (as in sweat) with the maximum vitamin C signal observed around 90 min after taking the vitamin pill. Note, however, the largely different current values (vs sweat), reflecting the significantly lower vitamin C tear concentration. A similar testing of the printed AAOx-biosensor strip toward saliva vitamin C assay is shown in Figure 4D(a). Such saliva measurements yielded a different temporal profile, with the maximum current response being observed 60 min after the intake of a 1000 mg vitamin C pill. Such different peak times are attributed to different metabolic pathways for the excretion of water soluble molecules from the plasma to different body fluids.[38] With the easy access to saliva and its promising application for on-the-spot decentralized testing, this biofluid was evaluated for additional vitamin C measurements. These saliva experiments were carried out 60 min after taking different amounts of vitamin C. The results, displayed in Figure 4D(b), show increasing current signals upon increasing the amounts of vitamin C pills. The largest saliva current response is observed 60 min after the oral intake of 250 mg vitamin C (Figure 4D(b)). Apparently, the increased response with larger amounts of vitamin C indicates no saturation point using small doses over the 50−250 mg range. However, the similar maximum currents observed in Figure 4D (a vs b for 1000 and 250 mg) indicate such absorption limits for high vitamin doses. Control experiments (similar to the sweat analysis) were performed for vitamin C in saliva, as shown in Figure S8. A negligible response is observed without the enzyme (Figure S8A) or without the pill intake (Figure S8B), reflecting the attractive performance of the enzymatic vitamin C sensor in this complex and the challenging biofluid. It should be highlighted that the collected saliva samples did not require any pretreatment, purification, or centrifugation. It is important to note that no contamination of saliva with pill or juice was observed as indicated, for example, from the temporal response of Figure 4D(a).

To apply the sensor onto the human body, the wearable sensor can be easily transferred onto the skin as an adhesive patch (Video S1). A piece of thin double-sided tape is cut via a computer-guided cutting machine, where two circular openings are made to expose the electrode. The cut double-sided tape can thereafter be applied onto the screen-printed sensor. Before use, the protective layer on the double-sided tape will be removed to expose the tape, and the sensor can be applied onto the skin (Figure 5A). The applied sensor is highly conformal to the skin surface and can be squeezed and twisted without delaminating from skin surfaces (Figure 5B). As a highly conformal sensor that can adapt to the curvilinear surfaces of the human body, the sensor is required to endure various deformations that occur on skin surfaces. To test the mechanical stability of the flexible sensor, a series or repeated bending and stretching deformations was applied to the sensor, and its chronoamperometric response was compared with the data generated from the pristine, undeformed sensor. Figure 5C (bottom) compares such chronoamperometric current responses of the 0.1 M PBS blank solution and of a 500 μM vitamin C solution before (a) and after 50 repeated inward (b) and outward (c) 180 bending, and 50 repeated 10% stretching (d). These data demonstrate no discernable difference in the electrochemical performance after these repeated deformations, confirming the robust mechanical stability of the tattoo sensor under extreme strains expected during the daily activities of the wearer.

CONCLUSIONS AND OUTLOOK

We presented a new wearable bioelectronic platform capable of tracking the vitamin C concentrations and dynamics in noninvasive biofluids. Specifically, we were able to demonstrate an epidermal enzymatic (ascorbate oxidase) sensor for the noninvasive monitoring of vitamin C in stimulated sweat. The skin-worn sensor thus measures the temporal rise and fall of sweat vitamin C concentrations in different subjects taking supplement vitamin C pills or juices without the interference of other sweat constituents. Different subjects display different temporal profiles to a given amount of vitamin C, indicating the potential of the epidermal biosensor toward personalized nutrition applications. The new biosensor offers significantly higher selectivity compared to the nonenzymatic voltammetric detection of vitamin C. AAOx-biosensor strips were also shown to be extremely useful for detecting changes in vitamin C levels in untreated saliva and tear samples collected at different times after taking vitamin pills. The use of a variety of control experiments along with specific enzymatic recognition clearly demonstrates that the response is solely because of the presence of vitamin C in these fluids. Such ability of wearable sensors to measure the dynamics of vitamin levels in noninvasive fluids represents an attractive approach for preventing nutritional imbalance and assessing adherence to vitamin intake. While demonstrating the proof-of-concept ability to use noninvasive epidermal sensors to detect variations in the vitamin C response, future efforts will focus on improving further the potential of this approach for personalized nutrition applications. These improvements will include: (i) the replacement of pilocarpine with long-term sweat generation drugs, such as carbachol, to facilitate continuous vitamin C monitoring in stimulated sweat using the same epidermal sensor over extended periods. This will be coupled with an extended lifetime of the AAOx biosensor. (ii) The integration of a sweat rate sensor to address potential

https://dx.doi.org/10.1021/acssensors.0c00604
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variation in the volume of the generated sweat. Our future work will involve a large-scale validation study, using reference gold standard methodologies, (e.g., HPLC or ELISA), and numerous human subjects to establish the vitamin C sweat and saliva concentrations along with the plasma−sweat correlations following the intake of different supplements and beverages. These studies will facilitate the precalibration of multiple sensors from the same batch of printed electrodes. Ultimately, such noninvasive monitoring of vitamin intake and dynamics could lead to better informed consumers and provide a personalized nutrition feedback and influence positive dietary behavior change. Such behavioral change influence can be reinforced by the easy integration of this new conformal wearable nutrition platform with miniaturized wireless electronics (Video S2) for continuous data acquisition of the individual dietary profile.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssensors.0c00604.

Study of different metalized carbon inks to enhance vitamin C detection; optimization of SWV parameters for vitamin C detection; optimization of CA parameters and enzyme layer modification; optimization of iontophoretic parameters; photography showing the anode before and after sweat stimulation; reproducibility test; in vitro characterization of the enzyme and nonenzyme vitamin C sensor; and control experiments for disposable saliva strips (PDF)

Wearable nutrition tattoo transfer process (MP4)

Integration of the wearable nutrition platform with miniaturized wireless electronics (MP4)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support from DSM Nutritional Products, Analytical Sciences is acknowledged. J.R.S., A.A.K., A.A., and M.A.M. acknowledge support from CNPq (grant 216981/2014-0), Fulbright Egyptian Scholar Program (ay2019-2020), Pakistan Higher Education Commission (HEC; Award no. 1-8/HEC/HRD/2018/8205), and the US Agency for International Development in Egypt (USAID), respectively.

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ACS Sens. 2020, 5, 1804−1813