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Technical Note

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Tattoo-Based Noninvasive Glucose Monitoring: A Proof-of-Concept Study

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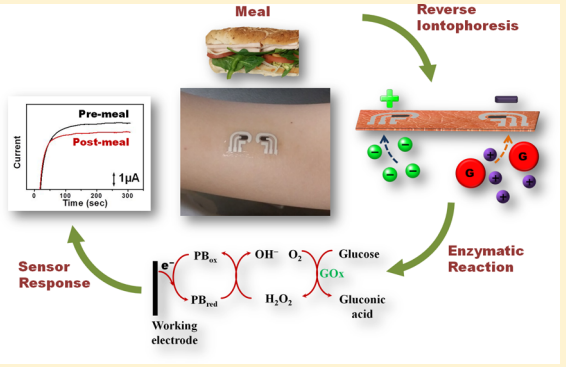
Supporting Information

ABSTRACT: We present a proof-of-concept demonstration of an all-printed temporary tattoo-based glucose sensor for noninvasive glycemic monitoring. The sensor represents the first example of an easy-to-wear flexible tattoo-based epidermal diagnostic device combining reverse iontophoretic extraction of interstitial glucose and an enzyme-based amperometric biosensor. In-vitro studies reveal the tattoo sensor's linear response toward physiologically relevant glucose levels with negligible interferences from common coexisting electroactive species. The iontophoretic-biosensing tattoo platform is reduced to practice by applying the device on human subjects and monitoring variations in glycemic levels due to food consumption. Correlation of the sensor response with that of a commercial glucose meter underscores the promise of the tattoo sensor to detect glucose levels in a noninvasive fashion. Control on-body experiments demonstrate the importance of the reverse iontophoresis operation and validate the sensor specificity. This preliminary investigation indicates that the tattoo-based iontophoresis-sensor platform holds considerable promise for efficient diabetes management and can be extended toward noninvasive monitoring of other physiologically relevant analytes present in the interstitial fluid.



Diabetes is one of the most widely spread modern lifestyle diseases affecting hundreds of millions of people and is among the leading causes of deaths globally.[1,2] Frequent monitoring of glucose is essential for optimal management of the disease and avoiding its associated problems.[3] Extensive research has led to the introduction and widespread use of self-testing blood glucose meters.[4] However, such self-testing methods rely on inconvenient and painful blood sampling from the finger tip that compromises the patient's compliance. Efforts aimed at addressing this drawback have resulted in several commercial continuous glucose monitoring systems. These enzyme-based microneedle sensors are inserted under the skin to measure glucose levels in the skin interstitial fluid (ISF) fluid.[5,6] Such minimally invasive sensing methods are based on the correlation between glucose levels in the ISF and in blood.[7,8] Completely noninvasive glucose sensing systems are highly desired to address the limitations of these subcutaneous systems (e.g., fingerstick validation, biofouling, microbial infection, and frequent replacement) and are thus ideal for diabetes management.

Extensive efforts have thus been aimed at developing noninvasive glucose sensors that rely on optical, spectroscopic, ultrasound, heat, electrical, or electrochemical techniques.[9,10] Among these, electrochemical techniques have shown the greatest promise.[4] Cygnus Inc. introduced the GlucoWatch electrochemical glucose sensor for noninvasive glucose monitoring.[11] This platform relied on the reverse iontophoresis technique to extract ISF glucose to the surface of the skin followed by the detection via an enzymatic electrochemical glucose sensor. Reverse ionotophoresis involves applying a mild current to the epidermis causing ions to migrate across the skin and toward the electrodes.[11] Sodium ions are the major charge carriers due to the negative charge of the human skin at neutral pH. The migration of sodium ions from across the skin to the cathode leads to electro-osmotic flow of the ISF toward the cathode. During this ISF flow, glucose is also transported toward the cathode. Thus, this technique can be used for noninvasive monitoring of ISF glucose levels.[11] However, the device was later discontinued as patients experienced skin irritation. This limitation has been addressed recently by employing a lower current density for the glucose extraction in connection to new noninvasive reverse iontophoresis glucose sensors.[12−14] However, these protocols have either been carried out under in vitro conditions[12,13] or require off-site glucose detection.[14]

The goal of this Technical Note is to demonstrate a proof-of-concept skin-worn temporary-tattoo based noninvasive glucose monitoring platform coupling an amperometric biosensor with a reverse iontophoresis operation (Figure 1). Our team has recently introduced body-compliant wearable electrochemical devices based on temporary tattoos that combine highly favorable substrate-skin elasticity with an attractive electrochemical performance.[15−17] The devices have been successfully

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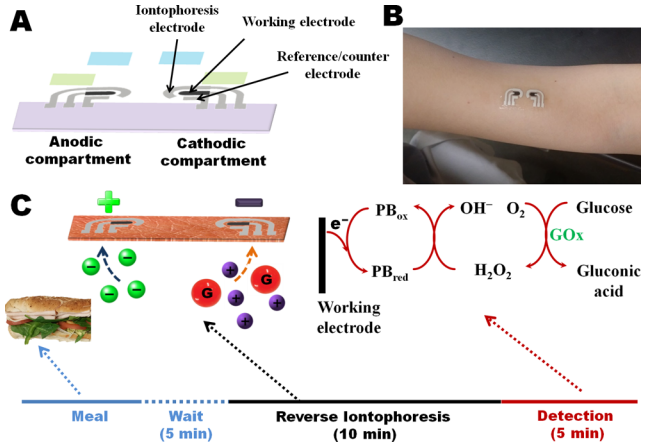

Figure 1. Tattoo-based platform for noninvasive glucose sensing. (A) Schematic of the printable iontophoretic-sensing system displaying the tattoo-based paper (purple), Ag/AgCl electrodes (silver), Prussian Blue electrodes (black), transparent insulating layer (green), and hydrogel layer (blue). (B) Photograph of a glucose iontophoretic-sensing tattoo device applied to a human subject. (C) Schematic of the time frame of a typical on-body study and the different processes involved in each phase.

applied for epidermal monitoring of sweat electrolytes (such as sodium)[18,19] and metabolites (such as lactate).[20] The new skin-worn tattoo-based glucose detection system uses a lower current density to extract the ISF glucose followed by selective amperometric biosensing using a glucose oxidase (GOx)-modified Prussian Blue transducer at a low potential as compared to GlucoWatch. Such flexible, low-cost, and aesthetically pleasing iontophoretic-biosensing tattoo platform can be easily mated with the human skin with the least level of intrusion to the wearer's routine. To realize both extraction and sensing operations using such a printable skin-worn tattoo platform, additional Ag/AgCl reverse-iontophoresis electrodes (along with the agarose hydrogel coating) have been incorporated for efficient delivery of ISF close to the working and counter/reference electrodes (Figure 1A). The biocatalytic reagent layer was optimized for imparting the sensitivity needed for detecting low (micromolar) glucose concentrations in the extracted ISF[14] and high specificity in the presence of common interfering electroactive species.

Following the in vitro optimization and demonstration of the sensor sensitivity and selectivity, the tattoo-based iontophoretic-biosensing system was evaluated toward noninvasive glucose monitoring in human subjects and was validated by simultaneous blood fingerstick measurements using a commercial glucose meter. The specificity of the tattoo GOx sensor was validated by applying it simultaneously with an enzyme-free tattoo sensor (no GOx control) on human subjects. The requirement of performing reverse iontophoresis prior to detection was demonstrated by analyzing the sensor response with and without active extraction of glucose ISF toward the sensor surface. These proof-of-principle on-body demonstrations reveal that the tattoo-based iontophoretic-biosensing platform holds considerable promise for noninvasive glucose monitoring in real-life situations. The attractive features of the new skin-worn system also highlight its potential for on-body monitoring of other target chemicals present in the interstitial fluid.

EXPERIMENTAL SECTION

Reagents and Instrumentation. Glucose oxidase (GOx) from *Aspergillus niger*, Type X-S (EC 1.1.3.4), chitosan, bovine serum albumin (BSA), sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), D(+)-glucose, L(+)-ascorbic acid, uric acid, acetaminophen, and agarose were obtained from Sigma-Aldrich (St. Louis, MO). Acetic acid was obtained from EMD Chemicals Inc. (Gibbstown, NJ). All reagents were used without further purification. Electrochemical characterizations were performed at room temperature using a CH Instruments electrochemical analyzer (model 1232A, Austin, TX) and PGSTAT 101 from Metrohm Autolab (The Netherlands).

Tattoo Fabrication, Modification, and Transfer Process. The fabrication process of the glucose tattoo was similar to our earlier work.[18−20] Briefly, sensor patterns were designed in AutoCAD (Autodesk, San Rafael, CA) and outsourced for fabrication on stainless steel through-hole 12 in. × 12 in. framed stencils (Metal Etch Services, San Marcos, CA). Papilio temporary transfer tattoo base paper was purchased from HPS LLC (Rhome, TX). A sequence of the silver/ silver chloride (Ag/AgCl) ink (4001, Engineered Conductive Materials, LLC, Delaware, OH), Prussian blue conductive carbon (C2070424P2, Gwent Group, Pontypool, U.K.) and insulator (Dupont 5036, Wilmington, DE) inks were patterned on the substrate employing an MPM-SPM semiautomatic screen printer (Speedline Technologies, Franklin, MA). As illustrated in Figure 1A, the tattoo sensor design consists of a pair of reverse iontophoresis electrodes (Ag/AgCl ink), a pseudo reference/counter (Ag/AgCl ink), and working electrodes (Prussian Blue ink). A transparent insulator was screen printed over the surface of the electrode pattern to confine the electrode and contact areas. The Ag/AgCl ink was cured at 130 °C for 3 min, while the Prussian Blue ink was cured at 80 °C for 10 min in a convection oven.

Following the printing of the tattoo electrode transducers, the working electrode was functionalized with the reagent layer. The enzyme GOx solution (34 mg/mL containing 10 mg/mL BSA stabilizer) was mixed with chitosan solution (0.5 wt % in 0.1 M acetic acid) in a 1:1 v/v ratio. Subsequently, a 2 μL droplet of the above solution was casted on the electrode and dried under ambient conditions.

In Vitro Characterization. These studies were performed using a 0.1 M phosphate buffer (pH 7.0) solution containing 133 mM NaCl. The operating potential for the tattoo glucose sensor was selected by using cyclic voltammetry. The amperometric response was recorded after 1 min incubation in the sample solution, using a potential step to −0.1 V (vs Ag/AgCl) for 60 s. The sensor specificity was examined in the presence of relevant electroactive constituents, namely, 10 μM each of ascorbic acid, uric acid, and acetaminophen.

On-Body Glucose Monitoring. An agarose hydrogel, covering all the electrodes, was applied to the tattoo sensor. The hydrogel was prepared by heating a continuously stirred agarose solution (4% w/v) in 0.1 M phosphate buffer (pH 7) at 120 °C for 15 min. The solution was then cooled down to 60 °C, and 100 μL of the solution was casted on the sensor area to form a uniform hydrogel layer covering all the three electrodes of both the anodic and cathodic contingents. The epidermal biosensor evaluation was performed in strict compliance with a protocol approved by the institutional review board (IRB) at the University of California, San Diego. A total of seven

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consenting healthy volunteers (4 males and 3 females between the ages of 20 and 40), with no prior medical history of heart conditions, diabetes, or chronic skeletomuscular pain, were recruited for participation in the study. The subjects were requested to arrive at the lab in a fasting state. The epidermal studies comprised of transferring the tattoo sensor to the skin followed by applying a constant current of 0.2 mA/cm^2 between the two reverse-iontophoresis electrodes for 10 min to extract ISF to the surface of the skin and finally recording the amperometric glucose response at an applied potential of −0.1 V (vs Ag/AgCl) for 5 min. A current density of 0.2 mA/cm^2 was selected for reverse iontophoresis based on a preliminary on-body study which revealed that lower current densities resulted in a slow ISF glucose extraction and hence in a delayed sensor response. The reverse-iontophoresis/detection cycle was performed first in the fasting state followed by consumption of a carbohydrate-rich meal. Thereafter, each subject was requested to wait for 5 min before a similar reverse-iontophoresis/detection cycle was repeated to measure the postmeal sensor response. The entire procedure is shown schematically in Figure 1C. The crucial role of reverse iontophoresis was examined by analyzing the response obtained from two glucose tattoo biosensors (applied simultaneously to subjects) with and without reverse iontophoresis. The selectivity of the on-body sensor to glucose was evaluated using two tattoos, one containing the GOx enzyme while the other devoid of it, applied simultaneously on the subjects' deltoid. For each human trial, simultaneous fingerstick blood glucose measurements were performed using commercial glucose strips (Accu-Chek Aviva Plus) to establish the correlation between the response of the tattoo sensor and that obtained from the commercial glucose meter.

RESULTS AND DISCUSSION

Rationale for Tattoo Design and Enzyme Modification. The new iontophoretic-biosensing system requires a different electrode pattern that includes the iontophoretic electrodes, compared to the three-electrode design of earlier tattoo-based electrochemical biosensors.[20] Each glucose tattoo sensor consisted of the anodic and cathodic contingents (Figure 1A). Each contingent is comprised of an Ag/AgCl electrode that performed as a counter/reference electrode. A printable Prussian-Blue transducer was employed in view of its high selectivity toward hydrogen peroxide, the detectable product of the GOx enzymatic reaction.[21] Each contingent consisted of an additional Ag/AgCl reverse iontophoretic electrode which encompassed the working and the counter/reference electrodes for efficient extraction of ISF close to the working and counter/reference electrodes. During the reverse iontophoresis operation, glucose is extracted at the cathodic contingent,[11] and hence the working electrode of the cathodic contingent was modified with the GOx enzyme for selective glucose detection. Chitosan was utilized as a polymeric matrix for immobilizing the enzyme on the transducer surface. While performing reverse iontophoresis, care must be taken to ensure proper contact between the skin and the sensor for efficient glucose extraction and to avoid skin irritation. This requirement was satisfied by evenly coating a layer of biocompatible agarose gel on each contingent to cover all the electrodes. Preliminary on-body studies revealed that the absence of the hydrogel layer caused perceptible skin irritation and burning. However, applying the agarose gel to the glucose sensor circumvented this issue. This could be attributed to the enhanced electrical contact between the sensor and the skin offered by the gel. The resulting glucose tattoo sensor can be easily applied to the skin, adhering and conforming to the contours of the epidermis, similar to a typical rub-on temporary tattoo (Figure 1B).

In Vitro Studies. The glucose level in the ISF is in the same concentration range as that in the blood.[22] However, the concentration of the ISF glucose extracted via reverse iontophoresis is approximately two orders lower than that of the corresponding ISF glucose level.[14] Keeping this in view, the response of the new glucose tattoo sensor was evaluated over the 0−100 μM glucose concentration range (Figure 2A). These

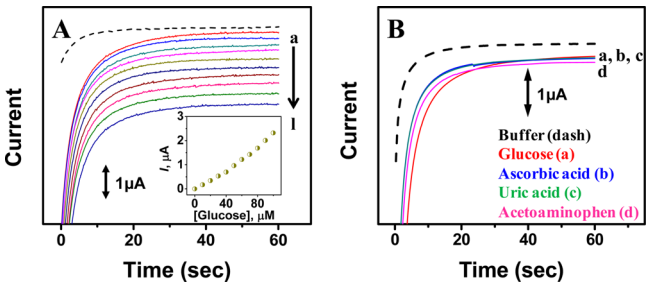

Figure 2. (A) Chronoamperometric response of the tattoo-based glucose sensor to increasing glucose concentrations from 0 μM (dashed) to 100 μM (plot "l") in buffer in 10 μM increments. (B) Interference study in the presence of 50 μM glucose (plot "a"), followed by subsequent 10 μM additions of ascorbic acid (plot "b"), uric acid (plot "c"), and acetaminophen (plot "d"). Potential step to −0.1 V (vs Ag/AgCl). Medium, phosphate-buffer with 133 mM NaCl (pH 7).

well-defined chronoamperometric responses to 10 μM glucose additions (Figure 2A, plots a-l) revealed that the sensor responded linearly and favorably over this range (sensitivity, 23 nA/μM; limit of detection, 3 μM), and could thus be utilized for detecting relevant ISF glucose levels extracted during on-body applications. Specificity of a sensor is of utmost importance for avoiding false alarms. Hence, the effect of physiologically relevant concentrations of common coexisting interfering electroactive species on the sensor response was examined. The results, displayed in Figure 2B, highlight the high specificity of the sensor toward glucose (Figure 2B, plot a) in the presence of ascorbic acid, uric acid, and acetaminophen (Figure 2B, plots b−d). Overall, the high sensitivity and selectivity demonstrated in Figure 2 reflect the coupling of the specific biocatalytic reaction with the low-potential amperomertic transduction at the Prussian-Blue transducer, as compared to the high detection potential utilized in GlucoWatch that could lead to compromised selectivity.[11]

On-Body Glucose Monitoring. After demonstrating in vitro the ability of the tattoo sensors to selectively measure micromolar glucose levels, we examined the on-body detection of ISF glucose levels in human subjects under real-life scenarios with the system worn on the skin. Meal consumption triggers a rapid rise in blood glucose levels that may lead to detrimental effects on diabetic patients. Hence, the present proof-of-concept study aimed at demonstrating the ability of the noninvasive tattoo sensor to monitor such sudden glycemic spikes. The first task was to identify the most appropriate time to perform the postmeal glucose sensing. Postmeal blood glucose levels of two subjects (1 male and 1 female) were thus measured at 10 min intervals over a 1 h period following a carbohydrate-rich meal (Figure S1 in the Supporting

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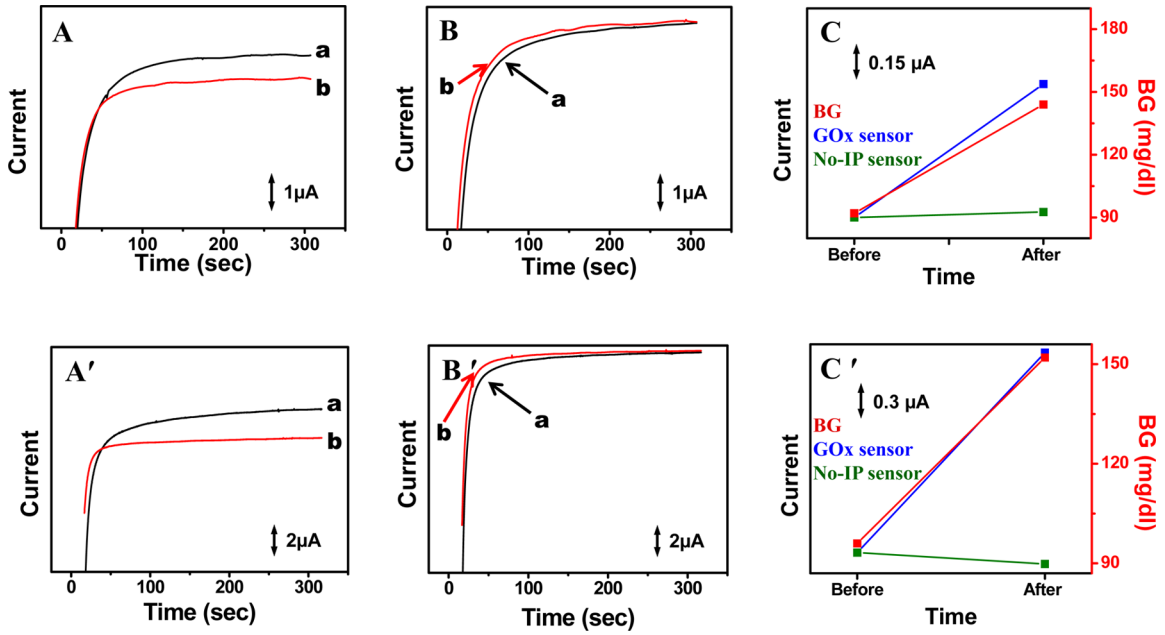

Figure 3. Amperograms obtained for noninvasive glucose detection obtained from two human subjects, wearing simultaneously the glucose tattoo sensor (A, A′) with and (B, B′) without the IP operation. (C,C′) Correlation between data obtained from tattoo biosensors, with and without the IP procedure, and that obtained using a blood glucose (BG) meter. Potential step to −0.1 V (vs Ag/AgCl).

Information). On the basis of these findings and literature data indicating an approximately 15−20 min lag time between ISF and blood glucose levels,[23] a 5 min waiting period (followed by 10 min of IP extraction) was considered for the postmeal glucose sensing. None of the subjects reported perceptible discomfort during these on-body studies. Only a mild tingling feeling at the skin under the iontophoresis electrodes was experienced by few subjects for less than 10 s at the beginning of the test.

Two important control experiments were carried out to corroborate the validity of the reverse iontophoresis-based glucose tattoo sensing system: (1) detection of passively diffused ISF glucose by a GOx-modified sensor (*No-IP* sensor) and (2) use of an unmodified (enzyme-free) sensor under active reverse-iontophoretic extraction of ISF (*No-GOx* sensor). Subjects were selected randomly to participate in each set of control experiments. For each subject, the control tattoo sensor was applied adjacent to a glucose tattoo sensor on the deltoid with a spatial gap of approximately 1.5 cm. The response of the control sensor was recorded in tandem with the glucose tattoo sensor.

Figure 3 displays data obtained from two subjects simultaneously adorning the glucose tattoo sensor and the *No-IP* sensor. It can be clearly noted that the respective glucose tattoo sensor displays a distinct increment in the postmeal current response (Figure 3A,A′ plot b) as compared to the fasting state (Figure 3A,A′ plots a). In contrast, the respective *No-IP* sensors show minimal change in the current response before and after the meal (Figure 3 B, B′ plots; a vs b). This study underpins the importance of active reverse iontophoretic extraction of ISF glucose for performing noninvasive glucose detection. Simultaneous blood glucose measurements using a commercial Accu-Chek Aviva Plus glucose meter and comparison with the response obtained from the tattoo sensors reveal the correlation between the noninvasive tattoo sensor and the blood glucose measurements.

Additional control experiments were carried out for other subjects wearing the glucose tattoo sensor along with a *No-GOx* sensor. In this set of studies, the response from glucose tattoo sensors was also significantly higher compared to that of the enzyme-free sensors, highlighting the specificity of the sensor to detect the glucose substrate in the presence of potential interfering species. Figure 4 displays a collection of

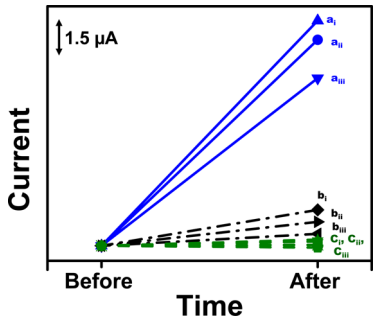

Figure 4. Combined data obtained from glucose tattoo sensors (plots "a_i", "a_{ii}", and "a_{iii}"), *No-GOx* sensors (plots "b_i", "b_{ii}", and "b_{iii}"), and *No-IP* sensors (plots "c_i", "c_{ii}", and "c_{iii}") before and after meal consumption. Conditions, as in Figure 3

amperometric signals recorded with the glucose sensors (plots "a_i", "a_{ii}", and "a_{iii}"), *No-GOx* sensors (plots "b_i", "b_{ii}", and "b_{iii}"), and the *No-IP* sensors (plots "c_i", "c_{ii}", and "c_{iii}") for different human subjects. These data clearly illustrate the ability of the tattoo sensors to detect spikes in the glucose level occurring due to food consumption. Another control experiment was performed to identify the variation in the sensor response in the absence of the glucose spike. During this control experiment, a glucose tattoo sensor and a *No-GOx* sensor were applied simultaneously to a human subject. It was noted that both the blood glucose level as well as the response from the two sensors remained fairly stable, thus underscoring

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the sensor's ability to specifically detect blood glucose spikes (data not shown).

CONCLUSIONS

This proof-of-concept study supports the application of a skin-worn tattoo-based wearable electrochemical biosensor for noninvasive glucose monitoring. The in vitro characterization of the tattoo sensors revealed their ability to detect micromolar levels of glucose in the presence of common interfering chemical species. On-body evaluation of the tattoo-based iontophoretic-biosensing platform further demonstrated the ability to detect the rise in the glucose level after a meal in a noninvasive fashion. Efforts are presently underway to build on this preliminary work to develop a tattoo-based biosensor for continuous noninvasive glucose monitoring. While key challenges remain toward such long operation, this preliminary proof-of-concept demonstration indicates the potential of the tattoo iontophoretic-biosensing platform for diabetes management. Future efforts are aimed at addressing these challenges and integrating the corresponding electronic backbone for powering the sensor, signal processing, and wireless communication on a flexible wearable platform and performing a large-scale glucose monitoring study. The new tattoo-based iontophoretic-biosensing platform could be readily expanded toward the noninvasive monitoring of other chemical markers present in the interstitial fluid and potentially for transcutaneous drug delivery.

ASSOCIATED CONTENT

Supporting Information

Plot depicting blood glucose levels measured for two subjects before and after meal consumption. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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