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AWARE: A Wearable Awareness with Real-time Exposure, for monitoring alcohol consumption impact through ethyl glucuronide detection

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ABSTRACT

Here we demonstrate for the first time a dynamic monitoring of the ethanol metabolite ethyl glucuronide (EtG) for a more robust evaluation of alcohol consumption, compared to conventional methods. A wearable biosensor device capable of reporting EtG levels in sweat continuously via low power impedance spectroscopy is reported. The custom hardware was compared against a conventional benchtop potentiostat, and demonstrated comparable results in the application of EtG detection in low volume sweat. The device successfully differentiated three distinct EtG concentrations correlating to simulated drinking scenarios estimated to be 1, 2, and 3 standard U.S. drinks consumed over a duration of 60 min, with $p < 0.0001$. This device has the potential to enable moderate drinkers to engage in guided decision-making, based on objective data, to address the needs of alcohol-sensitive populations. The device also will serve as a tool for researchers to better understand and characterize the relationship between sweat EtG and consumed alcohol.

Introduction

According to the 2015 National Survey on Drug Use and Health (NSDUH), 56% of people aged 18 years of age and older stated they had consumed at least one alcoholic drink during the previous month. In that duration, 27% of the same demographic reported engaging in binge drinking (4+ drinks for women and 5+ drinks for men). It makes sense that moderate drinking (1 drink per day for women and 2 drinks per day for men) falls between those percentages, highlighting its proclivity in U.S. society. Moderate drinking, though prevalent, does come with a host of concerns that warrants the personal monitoring of these events: physical risks can include direct consequences such as motor vehicle accidents during the period of intoxication, as well as influence on personal health such as increased risk of stroke, cancer, negative interactions with medication, impact on pregnant mothers, or mothers who are nursing (National Institute on Alcohol Abuse and Alcoholism, 1992).

To date, alcohol consumption monitoring devices typically report blood alcohol content (BAC) through correlation with either breath alcohol content (BrAC) or transdermal alcohol content (TAC) (Ashdown, Fleming, Spencer, Thompson, & Stevens, 2014; Leffingwell et al., 2013). The rapid expression of ethanol in breath and sweat is beneficial for intoxication monitoring. However, the rapid decay of expressed ethanol in these media can equally limit the application utility of these devices.

Alcohol is metabolized and excreted from the body by different processes or pathways (Kelly & Mozayani, 2012):

(1) Direct excretion of alcohol (5–10%) in urine, sweat, and breath.
(2) Metabolic conversion to acetaldehyde and further rapid conversion to acetate (>90%).
(3) Metabolic conversion to ethyl glucuronide (EtG) and ethyl sulfate (EtS), which is produced in the liver through the process of glucuronidation.

EtG and EtS represent a low total disposition of ethanol (~0.1%). They are readily expressed and detectable in transdermal

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human sweat (Cederbaum, 2012; Ramchandani, Bosron, & Li, 2001; Schummer, Appenzeller, & Wennig, 2008), making them promising candidates for wearable alcohol consumption monitoring, as shown previously by our group (Kinnamon, Muthukumar, Panneer Selvam, & Prasad, 2017; Panneer Selvam, Muthukumar, Kamakoti, & Prasad, 2016). Most of these excretory biomarkers are detectable in urine for a short period of time (~24 h). EtG, with its longer half-life, can be detectable in urine for up to 130 h for heavy drinkers (Helander, Böttcher, Fehr, Dahmen, & Beck, 2009). EtG is also present in blood/urine approximately 60 min after alcohol consumption. Fig. 1B shows the typical time course for ethanol and EtG expression in blood (Høiseth et al., 2007), which is highly correlated with expression in sweat. Here we propose that as an ethanol metabolite, EtG can potentially serve as an indirect indicator of alcohol consumption and metabolism with (compared to other direct markers) a prolonged detection window. Such a sensor would provide researchers a point-of-use tool to investigate non-invasively, and with more precision, the long-term impact of even light to moderate drinking in susceptible populations. Currently, there is not a robust tool for evaluating sweat EtG in these populations. The development of an EtG-based non-invasive biosensor may provide a long-standing quantitation of ethanol metabolism and its potentially harmful metabolites after the initial peaks of ethanol expression have subsided, which can improve the ability of susceptible populations to socially drink safely, while improving researchers' understanding of alcohol metabolites' impact on these populations.

With this vision in mind, we are demonstrating for the first time, a wearable biosensor prototype that has the potential to quantitatively monitor transdermal EtG. This device, also known as AWARE (AWARE: A Wearable Awareness with Real-time Exposure), has demonstrated the ability to detect cortisol in sweat and was adapted for EtG sensing (Sankhala, Muthukumar, & Prasad, 2018). The device uses electrochemical impedance spectroscopy (EIS) and antibody-based affinity capture assay built upon a disposable custom porous polymer test strip to quantify expression of EtG in human sweat. When a single alcoholic beverage (~14 g of pure ethanol) (Centers for Disease Control, 2017) is consumed, EtG will be expressed in sweat at an effective concentration in a given time (Schummer et al., 2008). We want to study the relationship between the EtG expressed in sweat with respect to the number of drinks consumed in a given time. However, many factors will affect these results, such as age, gender, weight/height, fitness, genetics, and other factors such as proximity of last meal that can all drastically modulate the rate of alcohol metabolism and affect how long it remains in the body after drinking (National Institute on Alcohol Abuse and Alcoholism, 1997; Zakhari, 2006). Before we take these factors into consideration, we need to ensure that our wearable biosensor prototype can distinguish different numbers of drinks in a laboratory setting. To do this, three different concentration ranges of EtG, based on a single alcoholic beverage in 1 h, have been designed to mimic common drink scenarios during moderate drinking (~14 g of pure ethanol) (Centers for Disease Control, 2017). Here, three different concentration ranges of EtG expression were evaluated: 13.3, 26.7, and 40 ng/mL, which, based on literature, are

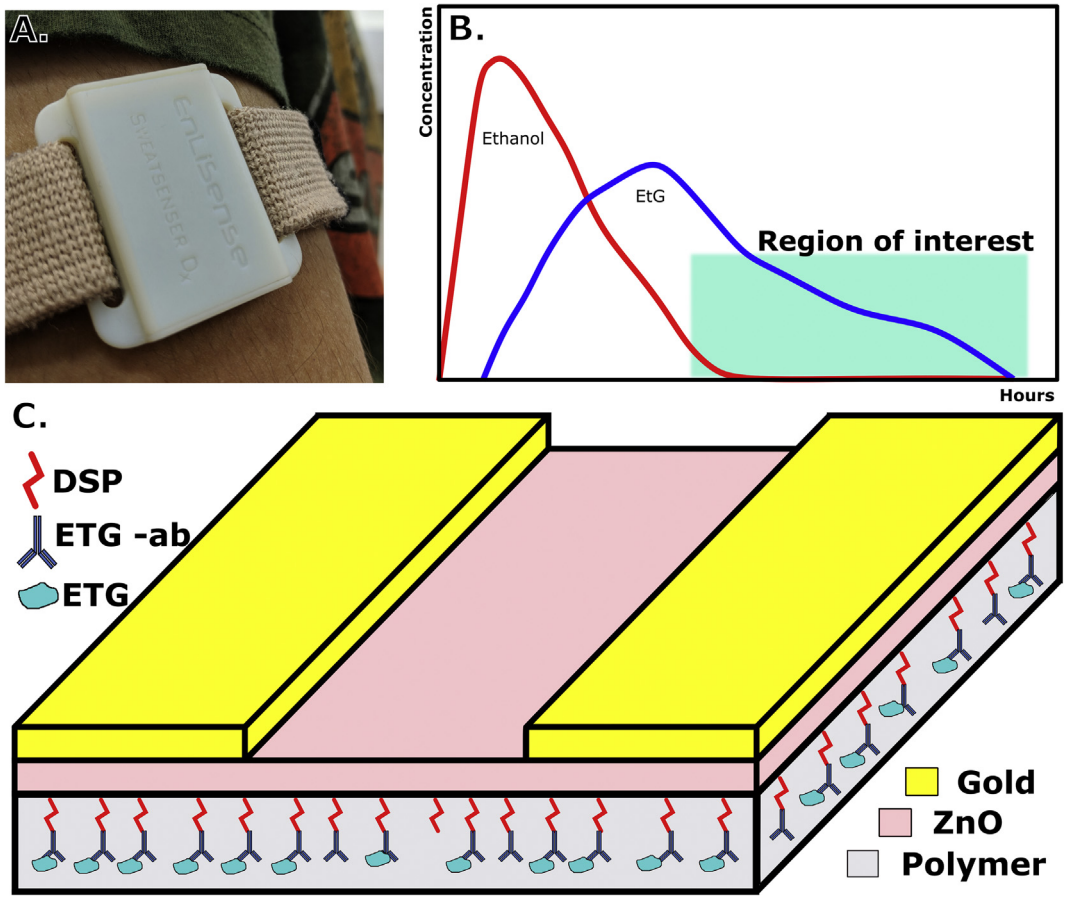

Fig. 1. A) Picture of AWARE device mounted on a test subject to show form-factor and wearability of the device. **B)** Graph showing typical time course expression of ethanol and EtG in blood. Adapted from real data (Høiseth et al., 2007). **C)** Schematic of the EtG affinity assay constructed on the porous polymer strip for disposable testing of EtG in sweat.

predicted to correlate to between 1 and 3 standard U.S. drinks (Høiseth et al., 2010; Jatlow et al., 2014; Schummer, Appenzeller, & Wennig, 2008).

Methods

Materials and reagents

The linker molecule Dithiobis [succinimidyl propionate] (DSP) and its solvent dimethyl sulfoxide (DMSO) were purchased from Thermo Fisher Scientific, Inc. (Waltham, Massachusetts, United States). Synthetic sweat prepared in the laboratory contained uric acid, lactic acid, ammonia, Na^+, K^+, and Cl^- ions. The composition for synthetic sweat was adopted from Mathew et al. (Mathew, Ariza, Rocha, Fernandes, & Vaz, 2008). Phosphate-buffered saline (PBS), and non-BSA tagged ethyl-β-D-glucuronide (EtG) were purchased from Sigma Aldrich (St. Louis, Missouri, United States), and monoclonal EtG antibody was purchased from EastCoast Bio (North Berwick, Maine, United States).

Sensor fabrication

Polymer substrates were patterned with conductive electrodes using a two-step shadow mask deposition. Shadow masks were laser-cut to a specified pattern using a Spirit Spectra-Physics laser manufacturing system. The electrode stack was composed of an initial semi-conductive ~125-nm layer of ZnO thin film pattern deposited using radio frequency magnetron sputtering. ZnO was deposited at 140 W for 60 min using 12-sccm argon flow. Twelve nm of conductive gold electrodes were patterned upon the ZnO layer. The gold on the ZnO sensor forms an ohmic contact between the ZnO film and the potentiostat.

AWARE device design

The AWARE device, shown in its wearable form in Fig. 1A, is adapted from the device shown in Sankhala et al. (2018). This device consists of four subsystems. Analog Devices ADuCM350 system-on-chip (SoC) was used to implement an impedance measurement loop and communicate with the other three subsystems. The Bluetooth communication was established using the Nordic nRF8001 Bluetooth chipset. This chipset allows for reporting and storing of data with a companion app on a smartphone or on a computer or smartphone, but for simplicity, the data were extracted via USB for this study. Texas Instruments HDC1080 was used to sense the environmental temperature and relative humidity. Texas Instruments BQ24040 was used to implement a USB/Li-ion battery power management system. The SoC consists of a configurable analog front end (AFE), which consists of a wave generator, a switch matrix, and a 2048-point discrete Fourier transform-based impedance analyzer. Two analog pins were selected, one of which acts as a generator and the other that acts as a sink. These pins are in turn connected to one of the sensors due to the action of the switch matrix. After a 13-msec DFT cycle on one sensor, the AFE switches to the next sensor channel. Thus, impedance measurement was performed on all four sensors in a combination sequence. In benchtop testing, this functionality was used to produce the multiple replicates, while in practice it will be used for redundancy. A photograph of the sensor affixed to a user is presented in Fig. 1A.

Affinity assay functionalization and protocols

Thiol-linker functionalization was carried out by dispensing a 3-μL volume of 10-mM DSP diluted in DMSO onto the ZnO sensing area of each tested sensor. Each sensor was incubated at room temperature for 3 h in darkness, followed by a 30-min incubation with 100 μg/mL of anti-EtG monoclonal antibodies diluted in PBS at room temperature. After successful functionalization of antibody, a 3-μL volume of synthetic sweat solution was dispensed onto the sensor strip prior to an EIS measurement. For calibration dosing responses, 3 μL of EtG doses (1, 10, 100, 1000, 10,000 ng/mL) prepared in synthetic sweat were added to the sensing region and allowed to incubate for 5 min before an EIS measurement was taken. Subsequent increasing concentrations were serially added and incubated following the same procedure to produce the calibration dose response. The described protocol is adapted from our group's previously published ZnO cortisol biosensor work, which was performed on a similar substrate (Munje, Muthukumar, Panneer Selvam, & Prasad, 2015). Volumes for the functionalization steps were maintained at or below 5 μL due to the effective volume of the sensor. EIS measurements were taken either by benchtop potentiostat (Gamry Instruments; Warminster, Pennsylvania, United States) or on the wearable AWARE platform. A low amplitude sinusoidal waveform (10 Hz–1 kHz) was used to conduct impedance measurements on both platforms. The impedance in all experiments was analyzed at 200 Hz, where a maximized 60° phase lag in the output impedance response was observed, indicating the capacitive nature of the sensor response.

Design drinking scenarios

In order to test the AWARE device in a more practical manner, sensors were continuously dosed at regular intervals with fixed concentrations of EtG in synthetic sweat that accurately correlate to the projected EtG composition of a person after consuming 1, 2, or 3 drinks in a single hour. Published studies on quantitative EtG measurements demonstrated that collected sweat from human volunteers using a PharmChek patch produced 0.0017–0.103 μg/mL of EtG when 38.0–154.6 g of equivalent pure ethanol were consumed, when analyzed using mass spectrometry (Schummer et al., 2008). According to the Centers for Disease Control and Prevention, USA, a standard drink in the United States is equal to 14.0 g (0.6 ounces) of pure alcohol (Centers for Disease Control, 2017). Based on the relationship between sweat EtG and ethanol in Schummer et al. (2008), it was calculated that a standard 14-g drink would be equivalent to approximately 24 ng/mL/hour of EtG in sweat, scaling proportionally with increased number of drinks, by linearizing the results. The correlation of EtG levels to consumed ethanol is an assumption supported by behaviors observed by Jatlow et al., 2014. Based on the conformation of the sensor, the maximum volume the sensor can hold is about 20 μL. To get a final concentration of EtG to 24 ng/mL on the sensor within a 1-h window of time, the total amount of EtG applied on the sensor should be 0.48 ng. This can be achieved by continuously dosing 3 μL of EtG 12 times over the course of an hour, at an effective concentration of 13.3 ng/mL of EtG in sweat. This relationship scales linearly with respect to number of drinks. So, 26.7 and 40.0 ng/mL of EtG were used to simulate 2 and 3 drinks in a given hour, respectively.

The sensors were tested at room temperature (23–25 °C) and ambient relative humidity (~45%). The experiment was broken into three regimens. In the first regimen, five replicates of EtG lacking synthetic sweat were applied at a rate of 3 μL every 5 min to the sensor in order to stabilize the EIS response. In the second regimen, 12 applications of 13.3 ng/mL EtG were applied at a rate of 3 μL every 5 min. In the last regimen, another five replicates of EtG lacking synthetic sweat were applied to the sensor at the same rate to represent stoppage of drinking. The same protocol was followed for 2 and 3 estimated drinks, at 26.7 and 40 ng/mL concentrations in the second regimen, respectively.

Results and discussion

EIS calibration of EtG detection in synthetic sweat

Non-Faradaic EIS measurements were used to quantify the binding interactions of EtG antigen in synthetic human sweat to the EtG-specific immunoassay constructed upon the polymer sensor. Binding was transduced based on the capacitive changes that occur at the electrical double layer (EDL) using the non-Faradaic EIS technique. We have previously demonstrated this modality of sensing for single capture affinity assays in a number of supporting publications (Kinnamon, Ghanta, Lin, Muthukumar, & Prasad, 2017; Kinnamon, Muthukumar, et al., 2017; Munje et al., 2015; Munje, Muthukumar, & Prasad, 2017; Shanmugam, Muthukumar, Selvam, & Prasad, 2016). A schematic for this constructed EtG detection assay is presented in Fig. 1C. The resulting binding of EtG and modulation of the EDL is transduced by the resulting change in effective impedance of the sensor. Fig. 2 shows the percent of change in impedance of the sensor with respect to various dose concentrations of EtG in synthetic human sweat, captured at 200 Hz, and collected on both the benchtop instrument and AWARE platform. On the benchtop platform, the sensors demonstrated detection of EtG with signal above the noise in the range of 10–10,000 ng/L. There is a consistent change in impedance as the dosage concentration of EtG increases, manifesting as 20.8% ± 1.97% for 10 ng/mL, 30.4% ± 1.39% for 100 ng/mL, 34.8% ± 1.51% for 1000 ng/mL, and 37.7% ± 1.27% for 10,000 ng/mL. The error bars are the standard error of mean from at least six measurements per data point. The governing equation for the impedance response with respect to EtG concentration on the benchtop potentiostat can be described as:

$$\text{Change in impedance } (\%) = 2.42 \times \ln(\text{EtG ng/mL}) + 17.12 \quad (1)$$

The performance of the benchtop potentiostat was used as a point of comparison for the performance of the AWARE wearable biosensor device. The same protocol was tested on the AWARE platform at 200 Hz to demonstrate proof of translatability of the proposed EtG biosensor onto an economical and compact form. Fig. 2 also shows the percent change in impedance from the baseline measurement for the wearable device. The percent change with respect to baseline measurement was observed to be 20.0% ± 0.62% for 10 ng/mL, 27.5% ± 0.48% for 100 ng/mL, 31.8% ± 0.48% for 1000 ng/mL, and 35.9% ± 0.25% for 10,000 ng/mL. The error bars are the standard error of the mean from at least six measurements per data point. The governing equation for the impedance response with respect to EtG concentration on the AWARE wearable device can be described as:

$$\text{Change in impedance } (\%) = 2.51 \times \ln(\text{EtG ng/mL}) + 13.57 \quad (2)$$

When compared to the previous response on the benchtop instrument, these results show that the AWARE device is performing comparably to the conventional benchtop instrument exhibiting a negligible difference in logarithmic slope, and a modest and predictable ~3.5% offset difference in impedance. These results demonstrate that the AWARE device can be used with confidence in future studies as an accurate reporter of impedance change with respect to EtG concentrations.

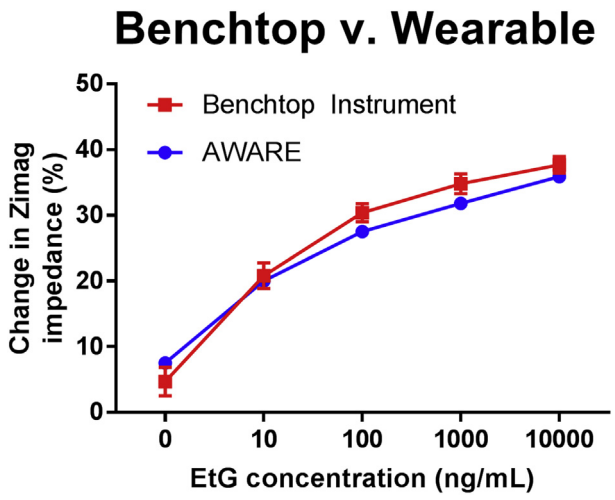

Fig. 2. Calibration dose response of EtG comparing the benchtop potentiostat and the wearable AWARE platform. Tested concentrations of EtG = 0, 10, 100, 1000, and 10,000 ng/mL of EtG. Error bars are standard error of mean. Error bars are not visible on all data points.

Continuous detection of EtG in three drinking scenarios

After the sensor had been validated for EtG detection, the AWARE device was tested in a more practical manner, simulating its ability to distinguish three different simulated drink scenarios from one another to demonstrate the technology's ability to quantitatively report, through sweat EtG, an indirect estimate of ethanol metabolism. Fig. 3A and Table 1 depict the continuous dosing of EtG to replicate the projected three drinking scenarios of 1–3 drinks per hour. With further deployment of our device, we may find that the relationship between EtG concentration and effective consumed alcohol. However, because the purpose of this work was to demonstrate the ability to quantify and differentiate various EtG dosages in a wearable form-factor, it was assumed based on cited literature that the relationship between EtG and consumed ethanol is a linear one at lower consumption levels (1–3 drinks), and that tested levels are in the physiologically relevant range even if they may be adjusted slightly in terms of the absolute number of drinks they correlate to.

Fig. 3B depicts the continuous response of EtG for the three different estimated drink profiles. Change in total impedance was calculated at 200 Hz with respect to baseline for all dose additions and was performed in triplicate. The results indicate that by the end of the first regimen, a total change in impedance of 359 Ω, 619 Ω, and 630 Ω was observed for 13.3, 26.7, and 40 ng/mL plots before the addition of EtG. In the second regimen, at the end of EtG dose application, the total change in impedance was 3458 Ω, 4327 Ω, and 5172 Ω for 13.3, 26.7, and 40.0 ng/mL of EtG, respectively. This shows that the sensor is relatively unresponsive to the addition of sample without the presence of EtG. The changes in impedance only came from the stabilization of the sensor. The sensor only responds in a discernible way after the EtG-containing sample is applied. Even more, the change in impedance corresponds with the estimated drink scenarios, increasing in magnitude of response with higher numbers of drinks. These results show that the sensor is responding to EtG binding at the electrode interface and is not responding to fluid loading on the device, which is an important distinction in electrochemical wearables. In the third and final regimen, the same protocol as the first regimen was followed, applying EtG-lacking sample. All three tested scenarios stopped responding after EtG was no longer present in the test sample, increasing by only 134 Ω, 127 Ω, and 74 Ω from the previous regimen for 13.3, 26.7, and 40.0 ng/mL, respectively, over the course of five additions. This reinforces that the sensor is only responding

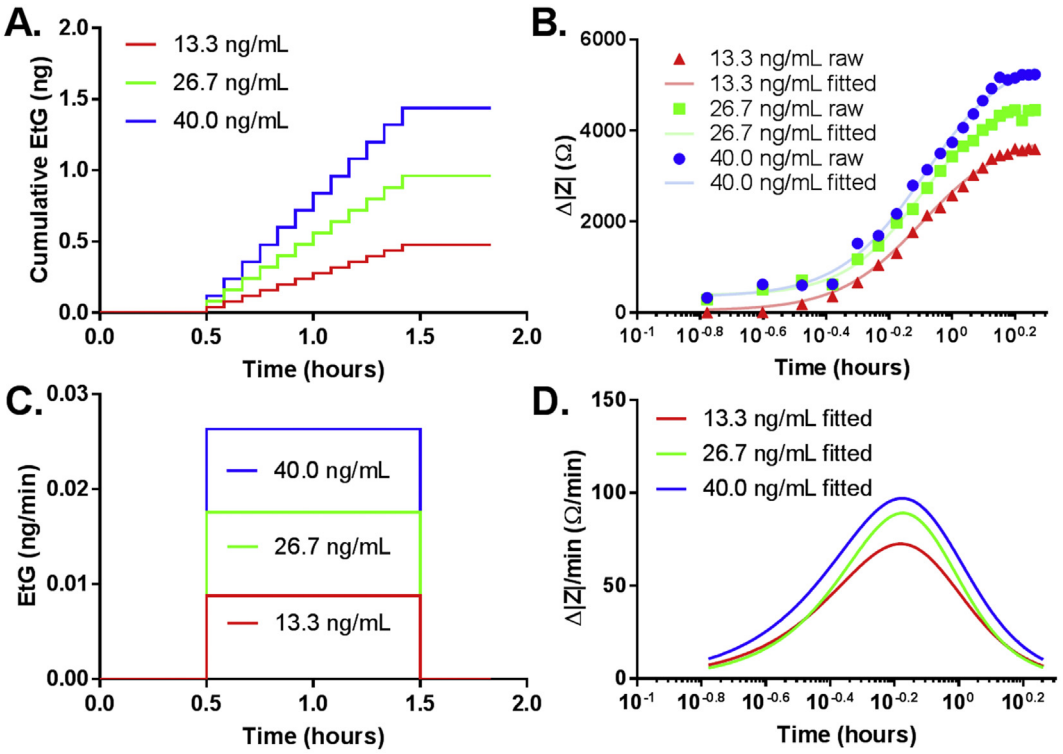

Fig. 3. A) Cumulative addition of EtG to the sensor by weight for each of the three test scenarios over the time course of the experimental protocol. **B)** Change in impedance response for the three test scenarios on the AWARE device. The raw data (points) were fitted with a logarithmic function (line). **C)** Addition of EtG to the sensor per unit time for the three test scenarios. The rate of change in impedance is derived from A. **D)** Change in impedance per unit time for the three test scenarios. The rate of change of the fitted lines in B.

to the EtG present in the applied samples. Using an unpaired *t* test to compare the five measurements from the third regimen, comparing each scenario with one another, a *p* value of less than 0.0001 was realized for all comparisons. This *p* value demonstrates statistical significance and motivating differentiation of the conditions. Although it is evident at the end of the experiment that each test scenario distinguishes itself from the others in a linear manner, as evidenced by the increasing plateau in impedance response in Fig. 3B, it is important to understand the dynamics of EtG expression in real time to give a more immediate feedback to the wearer, not only at the end of day. Fig. 3D represents the rate of change in impedance as a function of time for the data in Fig. 3B. The rate of change responds in a way that correlates well with the dosing response in Fig. 3B, where the three responses begin to differentiate only as dosing occurs, before returning back to the initial response as dosing ceases. Though it is clear based on the lesser degree of separation at the initial dosing steps that there will be a modest delay in the sensor's ability to differentiate EtG concentrations at a given time relative to what is physically present on the sensor, these results demonstrate an invaluable first step in achieving real-time detection of EtG in sweat. The implementation of analytical techniques may predict current EtG expression levels, based on the real-time sensor response compensating for this delay.

Table 1
Drink scenarios and associated dosing concentrations of EtG.

# Drinks/hour	Applied EtG/5 min (ng/mL)		
	0–25 min	25–85 min	85–110 min
1 Drink	0	13.3	0
2 Drinks	0	26.7	0
3 Drinks	0	40	0

Proposed implementation

The AWARE biosensor has the potential to become a commercial device for at-risk populations to better monitor the effects of their alcohol consumption, more precisely, before engaging in potentially harmful activities such as premature administration of medication. However, there is still much to be learned about the expression of sweat EtG as a function of consumed ethanol. First and foremost, this device will provide researchers with a tool to better evaluate that relationship in more detail, with the hope being that a device is realized that provides a more complete and quantitative indicator for ethanol expression and its potentially harmful metabolites to allow for improved decision guidance in prospective users.

The proposed device would function in the following way: The user would place the device on himself/herself with a fresh test strip. The user's pre-drinking baseline would be stored to calibrate the drinking session to their current sweat physiology. Based on the user's physiological metrics, and desired application of the technology, a predetermined “safe” EtG threshold(s) will be set to report to the user when that threshold has been reached. After the consumption of alcohol, the sensor will not immediately respond, as ethanol is expressed first. As ethanol starts to metabolize in the liver, EtG will gradually rise in expression before peaking after the peak of ethanol. The device will identify this increase in expressed EtG by evaluating the rate of change in impedance response to estimate the relative concentration of EtG in the sweat. When the level of EtG begins to decline, the rate of change will begin to reduce in magnitude as observed in Fig. 3D. The device will be programmed to determine when an acceptable level of EtG has been reached through the conversion of the rate of impedance change to EtG present in the user's sweat. It will take further

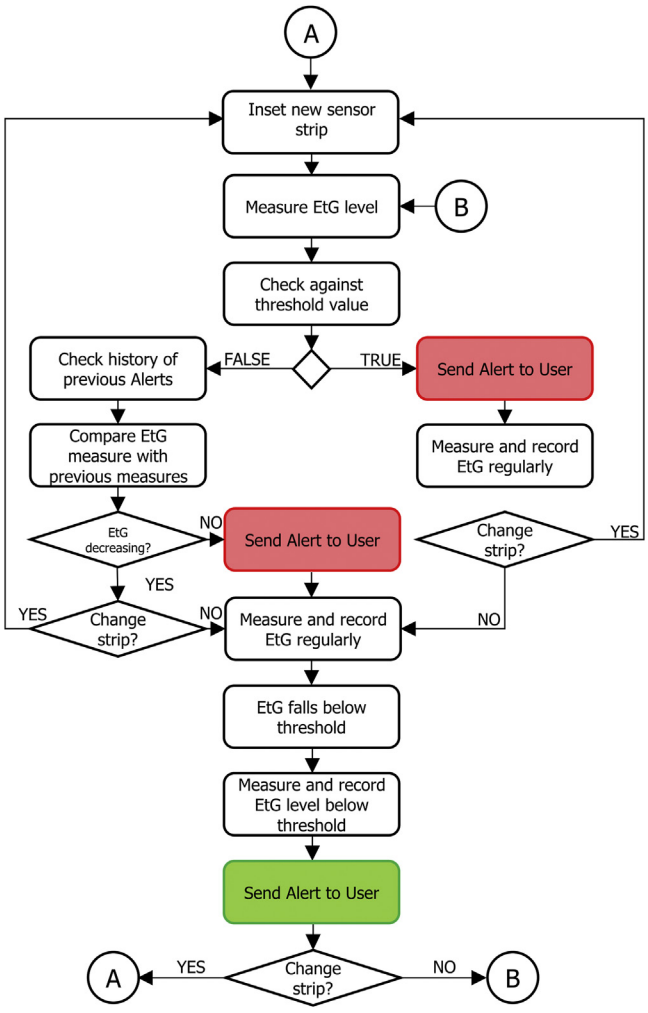

Fig. 4. Flowchart detailing the proposed functionality of the AWARE device, and the way it will decide when to alert the user of safe EtG metabolism levels to continue daily regimen.

investigation to determine these levels. To improve the accuracy of the device, it can be implemented in conjunction with current TAC monitors to provide a reference ethanol level for more accurate EtG calculations, as opposed to making it purely an alternative to TAC sensors. Fig. 4 highlights this proposed device functionality. The flow chart highlights the device-user interaction and the way it will report to the user when they are safely able to continue their daily regimen.

Conclusion

Here we demonstrate for the first time a wearable sensor that can differentiate EtG concentrations in synthetic human sweat, correlating to the predicted alcohol consumption profiles of moderate drinkers. The device performed comparably to conventional benchtop instruments. Three scenarios were tested for continuous monitoring of EtG, estimating the consumption of 1, 2, or 3 simulated drinks in a single hour. The device was not only able to detect and respond to EtG present in the samples, but also able to differentiate each test scenario from one another. It shows the ability for this device to act as an accurate reporter of EtG levels in low volume sweat, and its potential use to understand with more precision the disposition of ethanol and its metabolites after alcohol consumption, compared to standard BrAC and TAC measurements. This device could be deployed for use in populations at health risk from frequent moderate alcohol consumption. However, in the short-term the device provides a means for researchers to better understand the relationship between sweat EtG levels and consumed alcohol, which could serve as a much more robust indicator for the long-term impact of alcohol consumption compared to ethanol measurements alone. With further testing of this technology, it is feasible that this EtG assay could be implemented with other non-invasive samples such as urine, as our group has demonstrated previously for similar biomarkers (Kamakoti, Kinnamon, Choi, Jagannath, & Prasad, 2018).

Conflict of interest

Drs. Shalini Prasad and Sriram Muthukumar have a significant interest in Enlisense LLC, a company that may have a commercial interest in the results of this research and technology. The potential individual conflict of interest has been reviewed and managed by The University of Texas at Dallas, and played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alcohol.2018.10.006.

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