Introduction

The mobile health market is rapidly expanding and portable diagnostics tools offer an opportunity to decrease costs and increase the availability of healthcare. Here we present a smartphone based accessory and method for the rapid colorimetric detection of pH in sweat and saliva. Sweat pH can be correlated to sodium concentration and sweat rate in order to indicate to users the proper time to hydrate during physical exercise and avoid the risk of muscle cramps. Salivary pH below a critical threshold is correlated with enamel decalcification, an acidic breakdown of calcium in the teeth. We conduct a number of human trials with the device on a treadmill to demonstrate the ability to monitor changes in sweat pH due to exercise and electrolyte intake and predict optimal hydration. Additionally, we perform trials to measure salivary pH over time to monitor the effects of diet on oral health risks.

Methods

Overview of the system

The system presented here consists of a smartphone case, application, and test strips. The case has a slot in which a test strip can be inserted for colorimetric analysis using the
cellphone camera and a space to store up to 6 additional test strips. Fig. 1 shows a user removing a test strip from the storage space, collecting a sweat or saliva sample, and inserting the test strip in the device for analysis. The test strips incorporate 3 different elements inserted in a 3D printed support: an indicator strip, a reference strip and a flash diffuser. As an initial application presented here, we have designed the test strips in order to measure pH differences in sweat and saliva. The indicator strip consists of a 9 mm by 4 mm cutout of a pHydron Spectral 5.0 to 9.0 plastic pH indicator strip for sweat testing and a 1.0 to 14.0 strip for saliva testing. The reference strip is made of white plastic material and is used in order to detect changes in white balance on the iPhone camera due to different light conditions or user error. The flash diffuser consists of a 2 mm thick membrane of polydimethylsiloxane (PDMS). The purpose of the flash diffuser is to reduce variations in the reading for different lighting conditions and will be explained in the calibration section of this paper. It allows light from the smartphone’s flash to diffuse and illuminate the back of the tests strip uniformly. In addition, the case is 3D printed using opaque Vera black material in order to isolate the test strip from variable external light.

**Smartphone application**

To work with the accessory, we have developed a software app to handle image acquisition and processing, and data storage and manipulation. The app shown in Fig. 2 works as follows. First, upon loading the app, the user selects the test strip being used from a menu of different biomarker tests available, and the app loads the appropriate calibration data and user interface. Secondly, the user inserts the disposable test strip cartridge into the hardware accessory and touches the “Analyze” function on-screen. Thirdly, the app turns on the camera flash and takes an image of the test strip. The quantitative biomarker concentration is determined from the image (see “Colorimetric quantification” section) and displayed on screen in under five seconds. If pertinent to the specific biomarker, additional post-processing is also done from the biomarker results, and these results are displayed alongside the biomarker concentration. Finally, by swiping left, a scatter plot of the results is generated to show trends over time. If desired, the data set can also be sent via email for later viewing or additional analysis.

**Colorimetric quantification**

Although the ubiquity of smartphones with high-quality integrated cameras makes such devices ideal for point-of-care biomarker detection, the wide range of variations across different devices and of test strip illumination present significant challenges to accurate colorimetric quantification. Other investigators have addressed this problem by calibrating for ambient light conditions through conversion to color spaces which are less sensitive to changes in brightness.5,9 On
its own, this approach still requires uniform external illumination, and false colorimetric readings can be made if
the phone is not placed at the proper distance from the test strip.5 One of the unique opportunities of smartphone-based
colorimetric detection for portable diagnostics, however, is
that image acquisition can instead be automated, so that the
test strip is always held in the ideal position and imaged in the
same manner, and the data is not easily affected by deviations
in user protocol. Our device is isolated from ambient light with
the hardware accessory and diffuses light from the smart-
phone camera flash for reproducible and uniform illumina-
tion, improving measurement accuracy and minimizing the
potential for user error (see “Calibration” for details).

While the use of the camera flash instead of external light
removes much of the variability from the image acquisition,
there are additional steps which must be taken for accurate
quantitative processing. Although the image from the smart-
phone camera is initially defined with RGB (red green blue)
values, individual red, green, and blue channels do not
correlate well with pH over the range of a universal indicator
strip. Nevertheless, the RGB values can be readily converted to
an alternate color space that matches the color spectrum of
the test strips more closely. We chose to convert to hue, which
unlike RGB was found to monotonically increase with pH in
our experiments over the entire range of the colorimetric test
strips used, as can be seen in Fig. S1 of the ESI.1 This
advantage of the hue value over RGB for smartphone-based
colorimetric imaging has also been recently demonstrated by
Chang.10 In addition, recent work by Cantrell et al.11 has also
found hue to be more precise than RGB values for quantitative
analysis and less sensitive to variations in illumination,
making it an ideal candidate for our colorimetric test strip
analysis. After an initial calibration to determine the relation-
ship between the hue and the analyte concentration for each
test strip, this single hue value is sufficient to quantitatively
specify the color with a high degree of accuracy.

The process of image analysis is as follows. When the
“Analyze” button is pressed, the smartphone app activates the
camera flash, and an image is captured and stored first as an
RGBA (red green blue alpha) byte array. The alpha channel,
which is a measure of transparency, is discarded as it does not
vary with analyte concentration. The RGB array is split into two
sections—the first, corresponding to the upper colorimetric test
strip, and the second, to a lower reference region of known color
value which is used to compensate for variations between
different smartphone cameras and from automated camera
adjustment functions such as white balance. A 256 × 256 pixel
square is selected from the center of each of these sections, and
the hue value is calculated for each pixel from the RGB channels.
The hue values are sorted, and the median value is chosen to
minimize any remaining edge effects which are not removed by
the PDMS flash diffuser. Because the color of the plastic
reference section should not change between experiments if
the device works correctly, the image acquisition process is restarted
if the reference hue value varies from the expected calibration
value by more than 5. This serves to eliminate the possibility of a
user protocol error—if the test strip is inserted incorrectly and
the strip is not optically isolated, the reference check will fail and
the data will not be stored. If the reference check is passed, the
test hue value is converted into an analyte concentration by
means of a measured calibration curve and the relevant
biological information is displayed on-screen immediately. A
schematic of this process is given in Fig. 2d.

Calibration

The correlation between hue and pH is built into the
application, allowing users to run tests without additional
calibration. This is possible because the case is designed in a
way that minimizes the effect of external lighting as was
previously discussed. Fig. 3a illustrates the design of the case
around the camera and flash that allows for uniform lighting of
the test strip. The advantage of using this case design is
illustrated by the pictures of the strips in Fig. S2 of the ESI.† By
guiding the flash light through the PDMS diffuser on the strip
and behind the test strip, we avoid the need to build in a lighting
element, such as an LED, that would make the system bulkier
and require power input. The strip is imaged at a distance of 2.20
mm from the smartphone’s camera and the whole optical piece
has a depth of 4.90 mm. The relationship between hue and pH
for our test strips was established using buffer solutions and a
pH electrode (VWR SympHony SB70P) for an 8 point calibration.
Fig. 3b shows the variations in hue readings for 3 different
iPhones (models 4 and 4S). The same test strip and holder were
used at a given pH for all three iPhones meaning that the
differences can be attributed directly to differences in the
hardware of the iPhones. A third order polynomial was fitted
through the data points in order to obtain a correlation between
pH and hue. Fig. 3c shows the maximum deviation of the
measured pH from the calibrated values. It was found that the
variation between phones is the largest source of error, therefore
defining the accuracy of the system over the range of
physiologically relevant pH values to be within 0.2 pH units.

As explained previously, in order to further improve the
accuracy of our system, we incorporate a white reference strip
on our test strip. A large variation in the hue value of the white
reference indicates a failed measurement, possibly from a
faulty or incorrectly inserted test strip. If the application
detects an abnormal hue value, it rejects the data point and
signals to the user to take another reading.

Although the system shown here was designed for and
prototyped on the iPhone 4 and 4S, it could easily be ported to
any other smartphone platform with a CMOS camera. Even if
there are systematic differences in camera function and
sensitivity between smartphones from different manufacturers,
these differences can be corrected by calibrating the hue-to-pH
conversion function once for each smartphone model used. If
the hardware accessory is re-designed to fit over the camera and
properly re-calibrated, the most important metric for determin-
ing the accuracy of the device should still be the variation
between several phones of the same model, as described above.

Results and discussion

Sweat monitoring for hydration and electrolyte loss

It is generally well-accepted that dehydration can negatively
impact performance during exercise,12 however over-hydrating
is similarly dangerous since it can lead to hyponatremia, a disorder resulting from abnormally low sodium plasma concentrations.13,14 Hyponatremia has resulted in deaths both in athletic competitions and on the battlefield,15 due to a disruption in the osmotic balance across the blood-brain barrier, resulting in a rapid influx of water into the brain. In addition, studies have shown the sodium concentration of sweat can be used to evaluate the risk of muscle cramps in athletes.16,17 There is currently no system for accurately monitoring hydration levels or sweat sodium concentrations during exercise. However, there have been several studies showing the correlations between sweat pH and sodium concentration, as well as pH and sweat rate.18–20 Therefore, by accurately measuring changes in sweat pH during physical exercise, we can determine an optimal hydration strategy.

In a recent paper, Curto et al.21 demonstrated continuous, smartphone-based sweat pH monitoring using a wearable microfluidic platform. A wearable colorimetric pH sensor that can be incorporated directly into clothing has some obvious benefits; there are, however, applications in which a discrete sensing system with disposable cartridges, such as the one presented here, could be more appropriate. The unique use of the diffused smartphone flash in our system allows for easy and consistent imaging, even in the absence of external light. Moreover, the image processing algorithm was designed to require minimum computational power and is performed in real-time directly on the smartphone, without the need to transfer and process the data on an additional computer. While a robust wearable sensor is likely more cost-effective than disposable strips for a single application, the same accessory presented here could also be used to measure various additional biomarkers in sweat, saliva, or urine, which could ultimately be more cost-effective for an end user than needing separate microfluidic devices for each desired application.

In order to test the system and observe pH changes during exercise we have performed several experiments with users running on a treadmill between 30 and 60 min. The goal was to show that pH variations during exercise are significant and can indicate something about the intensity of the exercise. The participants in this study were all males between the ages of 25 and 37 who run regularly and participate in competitive racing events. Fig. 4a shows pH variations at 3 different run intensities for one user. It shows that at high exercise intensities, characterized by an increased sweat rate, the pH reaches less acidic levels. Fig. 4b shows the variations in pH between 3 users running at the same speed of 6 mph for 30 min. The results indicate that different users exercising in similar ways would need to exert different levels of effort. Fig. 4c shows the effect of cooling down by drinking 20 fl. oz. Gatorade at 40 min during 60 min runs. This is expected since sweating is the body’s method to regulate its temperature.22 Drinking cold fluids decreases the sweat rate and affects the sweat pH.

Because sweat pH has previously been correlated to the concentration of sodium,18 the experimental measurements of pH presented here also give the approximate sodium concentration of the sweat at each stage throughout the run.

**Fig. 3** (a) Test strip imaging with the first image showing the insertion of the test strip and the second one showing a cross sectional view of the optical system. (b) Hue–pH correlation for 3 different phones. (c) Maximum deviations for three different iPhones from the measured calibration curve.
The sodium concentration is shown on the right y axis in all the experiments in Fig. 4. It has also been found that the concentrations of the constituent electrolytes in sweat vary proportionally with sweat rate.\textsuperscript{19,20} By combining these results, an estimate of both the sodium and water losses over time due to sweating can be obtained. Since different users respond to exercise in different ways, there can be no universal guidelines for hydration and electrolyte intake. Using this system to monitor pH and sodium concentration allows for a better estimation of the fluid and electrolyte intake needed to fully replenish losses.

Additional experiments were performed to monitor the variations in sweat pH in other athletes, such as hot yoga practitioners. Hot yoga is the practice of yoga in hot and humid conditions which allows for easy sample collection and analysis. The users involved in these test trials were all women between 21 and 49 who perform hot yoga more than once a week. The users were instructed to perform exercises at their own pace and hydrate at their will. In Fig. 4d we show variation in sweat pH for the 4 users. The user highlighted in red only started hydrating at the 10 min mark and maintained a constant exercise pace after, hydrating regularly before cooling off during the last 5 min of the trial. The test trials which lasted up to 70 min show the expected patterns of pH variations due to hydration and cooling down periods. The maximum variations were under 1 pH unit and the changes in pH are extremely sensitive to changes in user intensity and hydration. These test trials demonstrate the advantage and applicability of discrete sweat pH measurements in non-continuous forms of athletic activity. These sudden changes in pH can be easily monitored using the system presented here and analyzed directly on a smartphone.

**Saliva pH monitoring for preventing enamel decalcification**

Another important application for pH monitoring is in preventing enamel decalcification. There have been numerous studies describing the effects of pH on oral health, more specifically on the dissolution of calcium hydroxyapatite, the main inorganic component of dental hard tissues.\textsuperscript{23} It is believed that enamel decalcification occurs below a certain critical pH that has previously been experimentally determined to be around 6.2.\textsuperscript{24} More recent papers describe the critical pH as a function of calcium concentration in saliva.\textsuperscript{25}

Here we demonstrate the potential of our system to monitor changes in salivary pH through the day. Data from one user during a typical day, from early morning (at \(t = 0\) h) until night (at \(t = 16\) h), is presented in Fig. 5a. Periods of food intake resulted in a rapid increase in pH and are highlighted in blue on the plot. Ultimately, the objective of salivary pH monitoring is to enable people to estimate their risk of enamel decalcification and to make suitable changes in their diet. For this purpose, estimating the amount of time the salivary pH is above a certain value is important. Fig. 5b gives this data for 3 different users (all males between the ages of 25 and 37) during a typical day. Fig. 5c shows the impact of diet on saliva pH for one user. Dental research has shown that bacterial interaction with carbohydrates in the mouth results in the formation of acids.\textsuperscript{26} The variations between users are a result of differences in diet and exercise, as well as innate metabolic

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**Fig. 4** (a) pH and sodium concentration variations for runs at different intensities. (b) Variations between 3 different users running at 6 mph. (c) Effect of cooling down with intake of 20 fl oz of Gatorade 40 min into the run for one user. Sodium levels are derived from ref. 16. (d) Hot yoga trials showing pH variations for 4 users during regular exercise. Red line show variation for a user who started hydrating at 10 min and maintained constant exercise and regular hydration for the reminder of the trial.
differences. Changes in diet for each user, however, have a marked impact on the salivary pH over the course of the day. By monitoring the salivary pH of one user on an ordinary and carbohydrate-rich diet (Fig. 5c), we can see the effect of carbohydrate-induced acid formation on average salivary pH. The device thus offers the potential for personalized tracking of the effects of various lifestyle changes over time.

On a typical balanced diet, the user is above the critical threshold of 6.2 pH for nearly 100% of the day, but this drops down to only half the day on a carb-rich diet, resulting in a substantially greater risk of enamel decalcification. These results indicate that the effect of changes in diet can be accurately measured with our device, thus allowing users to make the necessary dietary adjustments in order to improve oral health.

**Conclusion**

In this paper, we have presented a smartphone accessory and app for monitoring pH in sweat and saliva. Sweat pH monitoring is important for proper hydration during physical exercise, while saliva pH monitoring can help users make changes in their diet to prevent enamel decalcification. We have shown through a series of human trials that our system can be used in the context of sweat testing in order to prevent the risk of dehydration and to improve performance during physical activity. In the context of saliva testing, it can be used to evaluate the impact of dietary changes on the risks of enamel decalcification and overall oral health. Because the device presented allows for reproducible colorimetric detection with minimal user error, we believe that such a system could also be extended to a wide range of biomarkers for other portable diagnostics applications.

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