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Real-time measurement of human salivary cortisol for the assessment of psychological stress using a smartphone

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ABSTRACT

We present a simple smartphone-based measurement system consisting of a smartphone, a holder, and a lateral flow immune strip. The smartphone camera and light source were used to read the colorimetric signal from the lateral flow assay. A smartphone application was written and installed onto the smartphone. Various concentrations of cortisol were successfully measured using the images captured by the smartphone. Measurement of human salivary cortisol was then demonstrated using the lateral flow assay and the quantitative analysis was validated with the smartphone. The system was further evaluated using human saliva, demonstrating an accurate and reproducible platform for rapid and point-of-care quantification of cortisol using a smartphone-based measurement system.

1. Introduction

Stress, one of the major causes of psychiatric disease, is a physiological, physical, and emotional response to an external or internal stimulus such as social pressure, life-threatening experiences, or any of numerous diseases [1]. It can initiate further mental health problems such as depression, loss of confidence, and apathy, among others [1,2]. Cortisol is a well-known biomarker of psychological stress [2–4]. In general, current rapid and accurate diagnostic tools for measuring stress use interviews, an electroencephalogram (EEG), an electrocardiogram (ECG), body temperature, and a self-questionnaire [2,5]. Although such methods have demonstrated a reasonable sensitivity and resolution for physiological responses to external stimuli [2], they are relatively complex and require bulky equipment to perform the test, making them less suitable for personal use in public settings or for point-of-care testing (POCT).

There is no approach to point of care cortisol testing more practical than a lateral flow assay (LFA). The naked eye can analyze the test qualitatively without any special equipment but cannot do so quantitatively [6]. The measurement of psychological stress should also be performed in real time. We were able to create a personalized cortisol testing platform based on a smartphone application. It utilizes a sensitive colorimetric LFA strip for specific detection and quantification of cortisol in human saliva (Fig. 1). A simple LED is used to light the test strip and quantify its intensity. Complementary metal oxide semiconductor (CMOS) image sensors are used to process health-related data through digital signals embedded in a smartphone. These cortisol LFA strip images are detected in real-time and data readings are taken by converting the red, green and blue signal data to hue (H) and brightness values. A curve-fitting method was used for quantification. In this paper, we describe a simple method exploiting a smartphone to achieve real-time measurement of human salivary cortisol in combination with a lateral flow immune-strip.

2. Materials and methods

2.1. Preparation and operation of a smartphone-based measurement system

In order to analyze the test bands on the LFA strip, a smartphone colorimetric reader was prepared, which consists of three parts: smartphone, holder, and lateral flow immune-strip. The smartphone holder was designed using Solidworks 2010 (Solidworks Corporation, Concord, MA, USA) to block out all light except for that of the smartphone's flash and printed with a 3-D printer. The holder is responsible for accommodating the smartphone and the strip (Fig. 1). The smartphone holder dimensions are 70 × 90 × 130 mm. The smartphone fits into the front side of the smartphone holder with the strip inserted into the back. The

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strip is placed in front of the camera, parallel to the smartphone itself. A 12 × 21 mm guide was cut off from camera and added to the strip line. Light source emitted from the camera flash passes through the guide and reflects back to the camera lens. The smartphone focal length is 70 mm, so the length between the smartphone camera and the strip is 70 mm. The smartphone (Galaxy Note 1, SHV-E160S, Samsung Electronics Co., Korea) is equipped with a white LED and an 8-mega-pixel CMOS image sensor. The LED illuminates the paper strip, and the reflected light from the sample is measured inside the holder as discussed above.

A lateral flow immune-strip was developed for salivary cortisol and a cortisol standard solution was acquired from Cayman Chemical Co. (Ann Arbor, MI, USA). Briefly, the lateral flow immune-strip comprises a sample pad, a conjugate pad, a nitrocellulose membrane, and an absorbent pad. The anti-cortisol strip is based on a competitive assay [7], since the molecular weight of cortisol is too small to apply a sandwich assay. The reference line on the nitrocellulose membrane is made of cortisol–BSA and the test line is made of goat anti-mouse IgG. The coated membranes were dried for at least 2 h at 37 °C and the remaining membrane protein-binding sites were blocked by adding 1% BSA. After drying for 1 h at 37 °C, the assembled strip was cut into 5 mm widths and stored at 4 °C in a sealed polythene bag until use. After various concentrations of cortisol solution were applied to the strip, the holder was attached to a Galaxy Note 1 smartphone and our Android application program (see below) was initiated. When the strip was inserted into the smartphone holder, the test band was automatically detected. The application acquired an image, processed the data, and displayed the results in ng/ml.

2.2. Android-based smartphone application

Our smart application (“app”) measures the light scattering of colorimetric assays and digitally converts images captured by the smartphone into precise cortisol concentrations from human saliva samples. In order to quantify the LFAs with a smartphone, we implemented an app, termed a “Smartphone Linked Stress Measurement” (SLSM). The application operates as follows:

(1) A user is presented with the application menu (Fig. 2), where the user can begin stress level measurement.
(2) Once the user chooses to measure their stress level, the application presents the user with instructions. The user first prepares the LFA strip and then adds 4 drops of saliva into the strip inlet and wait 10 min for the reaction to complete. The smartphone holder is then attached to the phone, and the strip is inserted into the holder.
(3) Touching the phone screen begins image acquisition and calculation of hue and brightness. The computed values are then compared against the calibration data to obtain the cortisol concentration. The result is displayed on the screen. For the computation, the measurement time is checked first, as the cortisol concentration from human saliva varies by time of day. Based on this information, an appropriate threshold value for cortisol concentration is set. If the measured concentration is beyond the detection range of the sensor (1–100 ng/ml), the software displays an error message. The acquired data can also be saved as either an ASCII or an image (JPEG) file. The saved measurements may also be sent to a doctor remotely.

2.3. Image acquisition and data processing

The images acquired with our device were processed using an Android application that we created using the Android software developer (Google Incorporated, Santa Monica, CA, USA). The algorithm creates a detection area of the strip image; it then locates the test line region and detects a boundary. The only light source used was that of the smartphone itself. We compared the changes in the hue and brightness values with cortisol concentration using our SLSM (Fig. 3A, B). The hue value showed the color value (0–360), where red, green, and blue are standardized as 0/360, 120, and 240, respectively. The brightness value indicated the relative lightness or darkness of a particular color, from black to white. We could confirm that the b value is well suited for strip to be used for the estimation of the analytes.

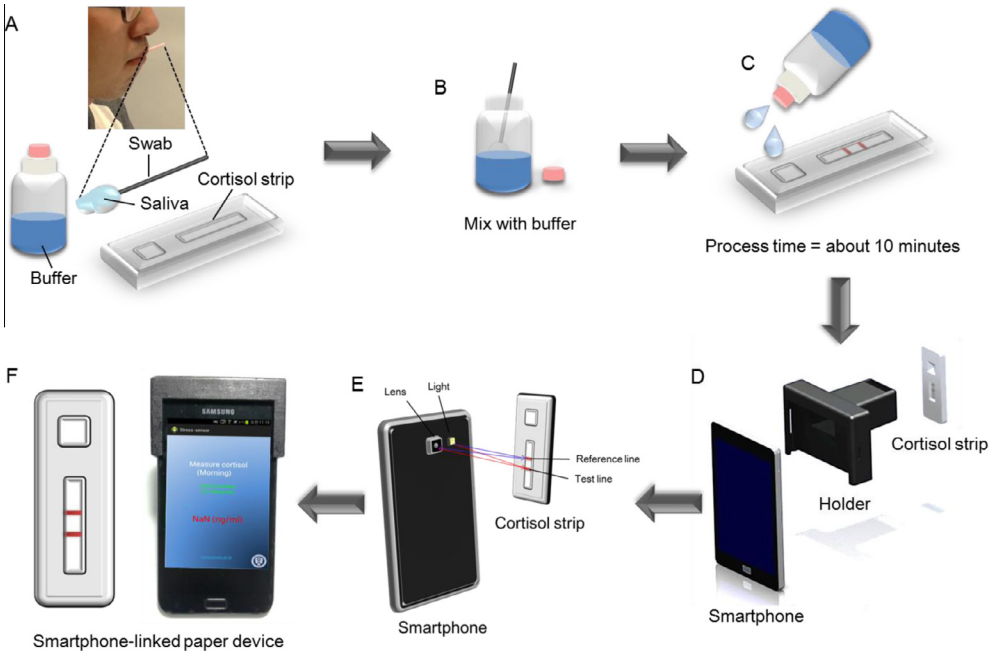

Fig. 1. A photograph and schematic diagram of the smartphone-based cortisol measurement system. (A and B) Saliva was collected using the swab and mixed with the buffer solution in a bottle. (C) Two or three drops of the buffer solution were loaded into the cortisol strip. (D) Exploded view of the complete system showing placement of holder and strip. (E and F) Smartphone-based reading system.

Fig. 2. Screenshots of our application running on an Android smartphone. (A) Once the application runs (B–D) the user reads the testing protocol explained under the pictures. (E and F) Following the activation of the smartphone camera, the user can simply touch the screen to capture the images of the test line. The acquired images are rapidly processed on the smartphone to quantify the cortisol amount within the human saliva sample.

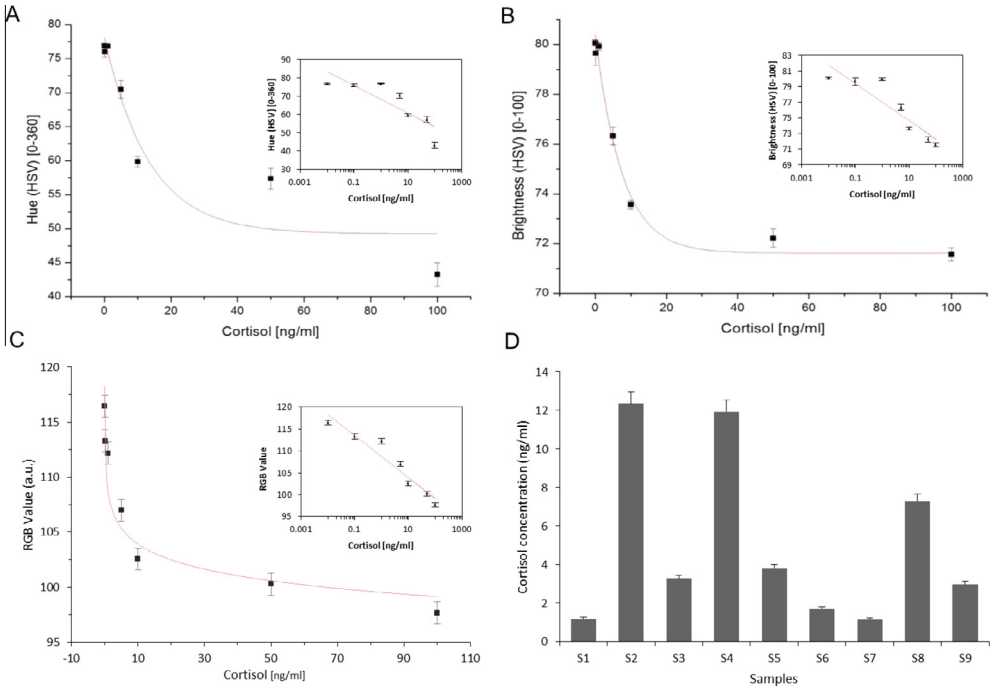

Fig. 3. Calibration curves for different calculations of (A) hue-HSV and (B) brightness-HSV. For the curve, seven different sets of calibration samples (0.01, 0.1, 1, 5, 10, 50 and 100 ng/ml) were measured. Error bars show the standard deviation for each dilution ($n = 20$). (C) The calibration curve was measured with the Image J software and used to obtained red–green–blue (RGB) value from the test strip. (D) Results from human saliva measured with the smartphone-based measurement system. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.4. Collection and preparation of human salivary samples

As shown in Fig. 3D, we investigated the performance of the strip using human saliva from volunteer members of our laboratory. The collected saliva sample's viscosity was decreased by holding the swab for 20–30 s in a dilution buffer. The prepared saliva samples were then used directly for the test. Two or three drops of the buffer solution were loaded into the strip. The intensities of the bands showing the cortisol concentration was measured by our smartphone-based measurement system. All measurements were conducted at room temperature.

3. Results and discussion

The color signals for the assays correlate well with the analyte concentration, as shown in Fig. 3. Our strip measurement system was first calibrated by testing known concentrations of cortisol (0.01, 0.1, 1, 5, 10, 50, and 100 ng/ml). The smartphone application was then calibrated with these samples. The data points and error bars in this figure represent the mean and relative standard deviation, respectively. The hue values were fit to an exponential decay curve of the cortisol concentration; the curve fit gave a confidence of determination (R^2) of 0.7967 (Fig. 3A). The responses are linear between cortisol concentrations of 0.01–10 ng/ml and the limit of detection is 1 ng/ml. When brightness values are used to form the cortisol concentration function, the modified exponential decay curve-fitting of the cortisol concentration data gave coefficients of determination (R^2) of 0.9837 (Fig. 3B). Therefore, we chose the calibration curve of brightness value as standard curve to validation the results. We also used Image J software (NIH, Bethesda, MD, USA) to measure the cortisol concentration at various concentrations (0.01, 0.1, 1, 5, 10, 50, 100 ng/ml). The linear regression equation from these measurements was $Y = -2.071\ln(x) + 108.69$, which yielded an R^2 coefficient value = 0.9276 (Fig. 3C). The responses are linear between 0.01 and 10 ng/ml cortisol, with a limit of detection of 0.01 ng/ml cortisol. To further evaluate our device, we collected saliva from our laboratory members to measure the cortisol level in human saliva. The buffered solution was loaded into the strip. The time of detection was approximately 10 min. We were then able to measure the cortisol concentration in our SLSM system. The data and error bars in this figure are the mean and relative standard deviation, respectively. Results from the human saliva samples were as follows: 1.2, 12.3, 3.3, 11.9, 3.8, 1.7, 1.2, 7.3 and 3.0 ng/ml (Fig. 3D).

4. Conclusion

We designed and manufactured a portable smartphone holder and cortisol LFA strip to produce a smartphone-based measurement system. Salivary cortisol concentration serves as a biomarker of psychological stress. Because physiological cortisol is secreted in a circadian rhythmic manner, the concentration of cortisol changes throughout the day [2,3]. Consequently, cortisol measurements should be carried out in real time. We successfully demonstrated that the color intensity of the test line was well correlated with the cortisol concentration in a range of 1–100 ng/ml when using the smartphone application. In the near future, a smartphone-linked measurement device will make it possible to analyze one's psychological state using a drop of human saliva. Our results have important implications for the development of smartphone-based biosensors for early diagnosis of human stress.

Conflict of interest

There is no conflict of interest.

Acknowledgements

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