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A Wearable Autonomous Colorimetric Sweat Induction System for Sweat Analysis

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Abstract— **The wearable biochemical sweat sensor's capability to provide insight into molecular information of health dynamics ignites sweat analysis as a promising noninvasive diagnosis scheme for precision medicine. Here, we demonstrate, for the first time, a colorimetric sweat induction microfluidic patch, which facilitates on-demand sweat glands activation by agonist coupled electrode and capillary action-based fluidics to collect microliter volumes (~5 µL) of sweat for monitoring its analytes by digital image analysis. The system's clinical utility demonstrated on a healthy volunteer for sweat pH monitoring flags the way towards other important sweat markers analysis for personalized healthcare.**

Clinical Relevance— **Sweat analysis based on wearable technologies and its correlation with blood analytes pave the way towards non-invasive point-of-care monitoring, as an alternative to blood analysis.**

I. Introduction

Over the past decades, wearable epidermal sensors have gained central attention in medical diagnostics, including personalized healthcare monitoring, real-time fitness analysis, and disease prediction. Although the interest in smart wearable technologies for electrophysiological parameters monitoring is continuing, recent advances in flexible technologies and the capability of integrating specific biological elements have empowered the development of wearable sensors for biochemical analytes monitoring from sweat [1]. Sweat comprises many relevant biomarkers such as ions, metabolites, proteins, and electrolytes, which could provide information on the health status of the body. For example, information about the electrolytes lost can be related to the dehydration level, the chloride concentration is used for cystic fibrosis screening [2], the pH value for metabolic alkalosis [3], and glucose for diabetics [4]. Many chemical species are actively exchanged between the sweat gland secretory coil and blood [2]. Even though sweat biochemistry is not entirely explored, analyzing sweat components and establishing a correlation between sweat and blood analytes creates an opportunity for a non-invasive clinical diagnosis tool alternative to blood through wearable platforms.

Several wearable sweat devices have been demonstrated in recent years based on different detection mechanisms. Among them, electrochemical detection, which is based on the change in potential or current upon the interaction with sweat analytes, is the most commonly used detection mechanism for sweat analysis. A multiplexed electrochemical device for simultaneous measurements of glucose, lactate, sodium, and potassium has been demonstrated for real-time health analysis of the subjects [5]. Also, low-cost electrochemical sensors based on printing technologies have been developed for simultaneous monitoring of sweat analytes [6]. Rapid advancement in wearable and flexible technology was exploited to integrate these electrochemical sensors into a skin tattoo and smartwatches for continuous sweat glucose monitoring [7, 8]. However, there are some critical challenges for the fabrication and the real-time application of electrochemical sweat sensors. First, the implementation of potentiometric and amperometry sensors involves complex circuits for the readout electronics. Also, electrochemical sweat sensors generally require preconditioning and calibration processes before real-time use to eliminate the baseline drift.

To address these limitations, simple colorimetric assays, which change the reagent color upon interaction with their respective analytes, have been developed. A soft microfluidic platform integrated with colorimetric-based detection technologies has been demonstrated for monitoring ions and metabolites. This technology has enabled the development of advanced capillary-based fluidic systems with additional features as chrono-sweat sampling for the time-variant monitoring of biomarkers [9]. Colorimetric assays-based devices have a rapid reaction time with minimal cross talk to interfering markers, and a simple readout without complex circuits. However, most of the developed colorimetric platforms are designed for sweat analysis for sports applications or in a sauna to increase the sweat rate. To address the challenges of non-invasive sweat monitoring on elderly people and patients, an active sweat stimulation system coupled with a colorimetric platform is required.

In this work, we present for the first time a coupled sweat gland stimulation system with a colorimetric patch, for autonomous sweat extraction and analysis. The patch's working mechanism is based on the activation of the sweat glands by stimulants for the sweat extraction, and analysis of its analyte concentration by estimating the change in color of a colorimetric assay through digital image analysis. Compared to the generally used cleanroom-based fabrication methods for colorimetric sweat patches, here, direct laser cutting is used to fabricate polymer-based microfluidic channels and paper-based colorimetric assay. The incorporation of a paper-based

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colorimetric assay facilitated the rapid capillary flow with the need of only a few µL volumes of sweat for the analysis. The facile implementation of the fabricated autonomous colorimetric sweat induction patch and its demonstration on a healthy subject for pH analysis can be easily adapted to other sweat markers analyses for the development of smart wearable sweat analyzers for point of care analysis.

II. Experimental Methods

A. Fabrication of Colorimetric Sweat Induction patch

The fabrication process of the colorimetric sweat patch consists of three stages: laser patterning of microfluidic features on polyethylene terephthalate layer (PET) and colorimetric assay paper, screen printing of sweat induction electrodes and coupling of the sweat stimulant gel, and vertical integration of all the layers to obtain the final patch (Fig. 1). In the first step, the microfluidic features such as the inlet for sweat intake, the collection region, and the outlet for sweat disposal were patterned using a CO_2-based laser cutter. Similar 2D patterns were fashioned on double-sided adhesive tape. The Whatman No.1 filter paper used for the coating of the colorimetric assay was also shaped in a similar laser design. For the sensor preparation, the pH sensor colorimetric assay was made with methyl red and bromothymol blue (ratio 1:3). The optimized assay was coated on a filter paper using a microneedle syringe and kept at room temperature for drying. For the fabrication of the sweat stimulation electrodes, a silver paste was screen printed on the PET foil and cured at 80° C for 20 minutes. The mask for fabricating the electrodes for sweat stimulation was also patterned by laser cutting the PET film. For the encapsulation, a biocompatible polyurethane (PU) layer was printed over electrodes and cured at 80° C for 1 hour. The stimulating gel was synthesized by dissolving agarose in deionized water and adding 1% pilocarpine as an agonist agent. The final colorimetric sweat patch was obtained by a 3D vertical integration of the top PET cover, the PET microfluidics, and the electrodes printed on the PET layer, using the laser patterned double-sided tape. The sweat stimulant gel was added to the electrodes by pouring the prepared transparent gel and solidifying it at 4 °C for 1 hour.

B. Colorimetric Sweat Induction Patch Sensing Mechanism

The colorimetric sweat induction patch mechanism consists of an active stimulation of the eccrine sweat glands by applying a constant electric current to trigger the stimulant from the gel-coupled electrode [10]. The electric current intensity and time duration were optimized to 1.3 mA for 5 min, which allows a sufficient electrode activation and agonist dosage to be delivered to activate the sweat gland. The electrode's activation causes repelling of the sweat stimulant from the gel, which will diffuses into the skin and attach to the acetylcholine accepters of the sweat glands to induce the sweat. The induced sweat was quickly drifted to the patch's inlet through capillary action and transported to the colorimetric assay-coated paper within a minute. The sweat interaction with the assay causes a change in the assay's color due to protonation depending on the sweat pH. The assay RGB index was assessed using an RGB color analyzer, a smartphone-based application, and converted to the color space parameters L*, a*, b* (with respect to the reference marker) to obtain the sweat pH value from the calibration [11].

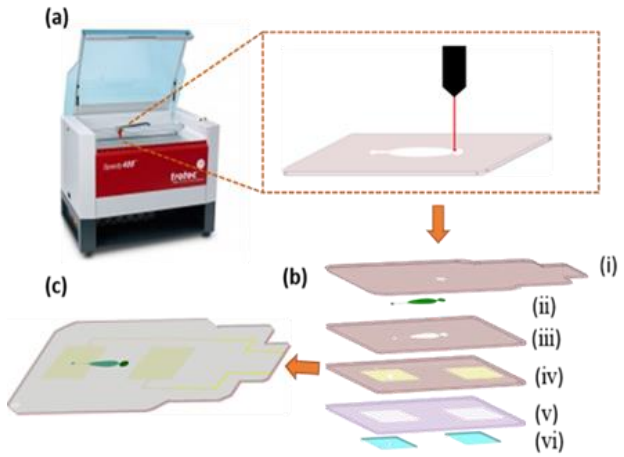

Figure 1. Schematic illustration of the non-lithographic device fabrication. (a) The features of each layer were designed with a CAD software and then laser cut. (b) Each layer is vertically assembled using patterned double-sided tape to get the final patch: (i) top layer, (ii) colorimetric assay paper, (iii) microfluidic layer, (iv) stimulation electrodes printed layer, (v) gel holding layer, (vi) stimulants gel coupling to electrodes. (c) The final fabricated colorimetric sweat induction system.

The principle of operation of the patch is illustrated in Fig. 2. The autonomous process of sweat gland activation and sweat analysis by the color change of assay eliminates the need for a multistep process and separate analysis tools. This generic colorimetric sweat induction platform can be further extended to an array of miniaturized sweat stimulation electrodes with multiple inlets for sweat intake. The fabricated sweat induction patch can be fully automated for on-demand colorimetric analysis or preprogrammed for timely activation. The incorporation of the multiple colorimetric assays corresponding to different sweat markers can facilitate the simultaneous monitoring of different analytes in a single platform.

C. Colorimetric Image Analysis

The optical images of the colorimetric assay were captured using a digital or smartphone-based camera, and the images were quantitatively analyzed by converting the extracted RGB values into color space values. The degree in relative difference ΔE^*_{ab} was calculated relative to a white reference to obtain a normalized value to determine the analyte concentration [12]. The normalization eliminates the effects of different light sources.

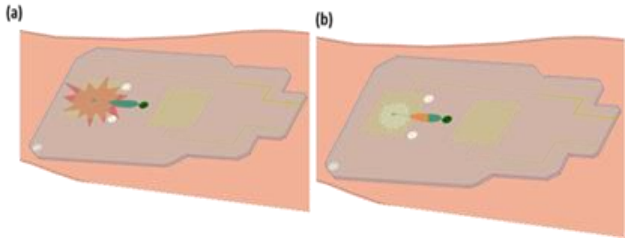

Figure 2. Schematic illustration of the colorimetric patch mechanism on a skin. (a) The activation of the electrode causes the delivery of sweat stimulant to stimulate the sweat gland and (b) the induced sweat collected by the patch causes a change of the assay color corresponding to the sweat analyte concentration.

ΔE^*_{ab} is defined as:

$$\Delta E^*_{ab} = [\,(L^*_n - L^*_x)^2 + (a^*_n - a^*_x)^2 + (b^*_n - b^*_x)^2]^{(1/2)} \quad (1)$$

Where L^*_n, a^*_n, b^*_n, and L^*_x, a^*_x, b^*_x represents the values at n^{th} assay location and white reference location, respectively. L^*, a^*, b^* parameters are defined as,

$$L^* = 116f(Y/Y_n) - 16$$
$$a^* = 500[f(X/X_n) - f(Y/Y_n)] \quad (2)$$
$$b^* = 200[f(Y/Y_n) - f(Z/Z_n)]$$

$$f(t) = \begin{cases} t^{1/3} & \text{if } t > (6/23)^3 \\ 1/3(29/6)^2\, t + 4/29 & \text{otherwise} \end{cases}$$

Under the illuminant D65, the values are equal to $X_n = 95.048$, $Y_n = 100.00$ and $Z_n = 108.884$. The conversion to the XYZ color parameters was accomplished by taking the averaged RGB value to eliminate the special non-uniformities in the captured color image.

III. Results and Discussion

Compared to the photolithographic technique and cleanroom resources based microfluidic fabrication, laser cutting required only a two-step process: a CAD-based designing of the microfluidic features and creating the PET's designed feature substrate using a laser cutter. The fabrication needed on the order of few minutes compared the photolithographic process, which required on the order of a few hours with a multistep fabrication process. The proper alignment of all the laser-cut layers in a vertical direction renders the final digitally manufactured integrated patch as shown in Fig. 3.

The use of a patterned double-sided adhesive layer eliminates the necessity of the oxygen plasma for bonding. The medical-grade adhesive is used to facilitate the adhesion of the patch to the skin. White reference markers included at the top of the device enable color balancing, for the accurate extraction under different light sources. This induction system coupled colorimetric platform enables autonomous sweat extraction and analysis within a few minutes.

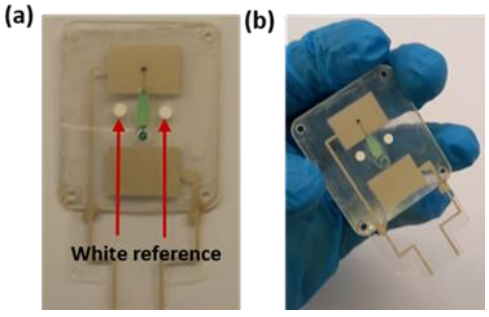

Figure 3. Optical images of the fabricated patch. (a) Top-view showing the white reference markers for color balancing and (b) the full colorimetric sweat induction patch.

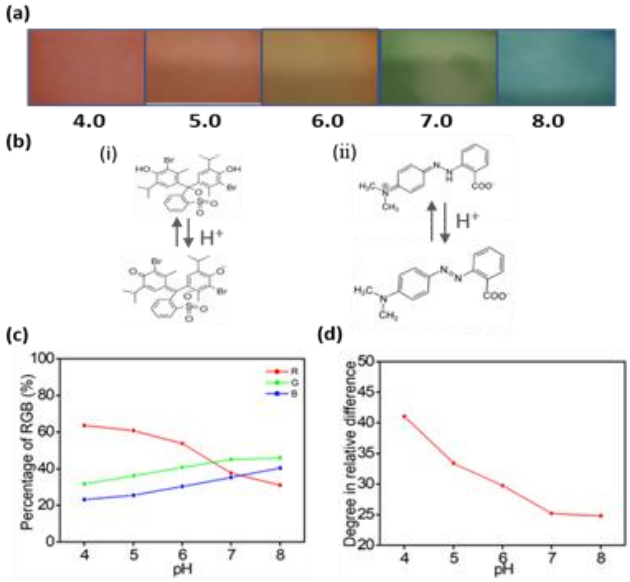

Figure 4. (a) Characterization of the pH assay over the range between 4 to 8, (b) protonation process in (i) methyl red and (ii) bromothymol blue, (c) relation between %RGB value extracted and corresponding pH, (d) degree in relative difference corresponding to pH.

Upon interaction with different pH, the assay has a color shift from red (pH 4) to green (pH 8). The assay composed of methyl red and bromothymol covers the sensing of the entire range of pH from 4 to 8, as shown in Fig. 4(a). The pH 4 to 6 was sensed by methyl red through its carboxylic acid and amine functional groups. Bromothymol blue was responsible for detecting the pH varying from 6 to 8 through triphenylmethane formation in the alkaline environment (Fig. 4(b)). The assay's color changes were captured by a digital camera and converted to a percentage of RGB color Fig. 4(c). The extracted RGB index was converted to the color space parameters L*, a*, b* to obtain the degree in relative difference corresponding to the pH value (Fig. 4(d)). The elimination of the different lighting conditioning was achieved by white balancing using the reference markers.

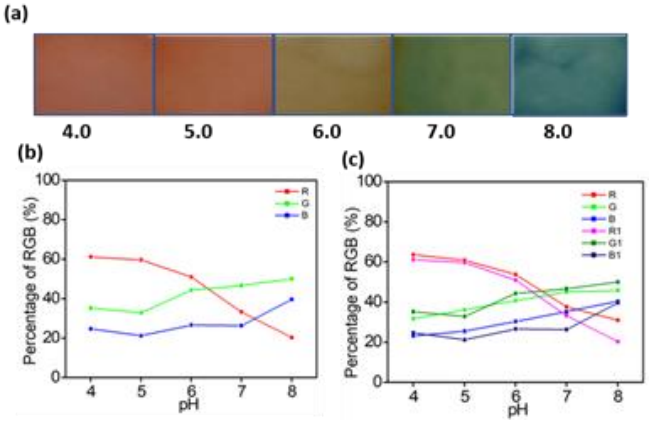

Figure 5. (a) Characterization of the pH in artificial sweat with varying pH from 4 to 8, (b) % RGB extracted from artificial sweat response, (c) comparison of % RGB from McIlvaine buffer (R, G, B), and artificial sweat (R1, G1, B1) response.

To further investigate the developed assay response to different analytes present in the sweat, the response of the assay was recorded in synthetic sweat with pH varying from 4 to 8 as shown in Fig. 5(a). The synthetic sweat was prepared with 6 mM KCl, 50 mM NaCl, 0.17 mM glucose, 10 mM urea, 5 mM NH4Cl, and 0.4 mM CaCl2 with varying pH. The percentage of RGB color was extracted and compared with the RGB index obtained from McIlvaine buffer solutions with its pH varying from 4 to 8 (Fig. 5(b,c)). The sensor assay exhibited nearly equal color change response in both McIlvaine buffer and artificial sweat, demonstrating the successful development of an assay for on-body real-time sweat analysis.

A. *On-Body Analysis Using Colorimetric Sweat Induction Patch*

The utility of the fabricated patch for autonomous colorimetric sweat analysis was demonstrated on a healthy subject for sweat pH analysis. On-body sweat studies were carried out in compliance with the protocol approved by the institute Human Research Ethics Committee (HREC No. 057-2019 / 07.10.2019). The on-body real-time sweat analysis was performed by interfacing the patch with the stimulation electronics tightly adhering to the forearm of the subject (Fig. 6(a)) [13]. The stimulation electrode was activated for 5 min, which efficiently delivers the stimulant for the sweat gland activation. The induced sweat was quickly taken into the inlet of the patch. The sweat interaction with the assay paper in the channel allows its fast transport towards the outlet. The change in color of assay due to the interaction of the sweat corresponds to its pH level (Fig. 6(b)).

The assay color's RGB index was assessed using a smartphone-based application and converted to color space parameters with respect to the reference marker. The pH value of the subject was extracted using the degree in relative difference calibration. The fabricated patch can be further miniaturized and automated by incorporating near field communication (NFC) technology, which facilitates wireless power transfer and data communication, including both automatic readout and sweat glands stimulation control through a NFC enabled smartphone or tablet. A custom-made android application can be easily implemented for the direct extraction of the sweat pH.

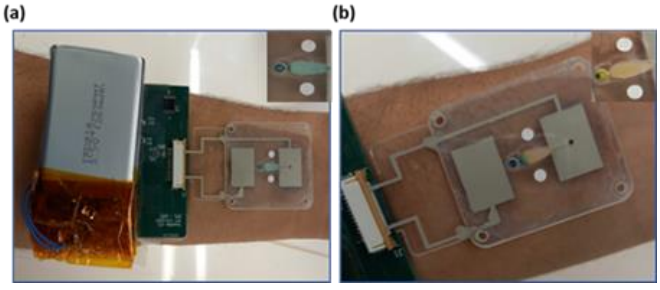

Figure 6: (a) Colorimetric sweat induction patch interfaced with the PCB and adhering to the subject forearm for sweat stimulation analysis. The insets show a color of assay before sweat interaction (b) the induced sweat interacted with the assay causing a color change corresponding to the sweat pH value due to protonation. Inset shows color of assay after sweat interaction (The pH of the subject was estimated to be ~5.5).

IV. Conclusion

In this work, we developed a wearable colorimetric patch for simultaneous sweat induction, sweat extraction, and sweat pH estimation. The effective monitoring of sweat pH was demonstrated directly on the skin of a healthy subject using the integrated platform. The presented fabrication scheme and integration process allow a simpler implementation of a colorimetric patch compared to previously reported multistep soft lithographic based colorimetric devices. This technology can be extended to the monitoring of multiple sweat analytes for smart healthcare applications.

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