TITLE:
A detailed protocol for perspiration monitoring using a novel, small, wireless device.

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KEYWORDS:
Perspiration monitoring, water vapor flux, sensor, silica gel, sympathetic activity, emotional sweating, data processing, transepidermal water loss

SHORT ABSTRACT:
Recently, we developed a small wireless device for perspiration monitoring. In this article, we present detailed protocols on how to use the device for perspiration monitoring with an example of the sympathetic activity test.

LONG ABSTRACT:
Perspiration monitoring can be utilized for the detection of certain diseases, such as

thermoregulation and mental disorders, particularly when the patients are unaware of such disorders or are having difficulty expressing their symptoms. Until now, several devices for perspiration monitoring have been developed; however, such devices tend to have a relatively large exterior, considerable power consumption, and/or less sensitivity.

Recently, we developed a small, wireless device for perspiration monitoring. The device consists of a temperature/relative humidity (T/RH) sensor, battery-driven small data logger, and silica gel as a desiccant in a small cylindrical exterior. The T/RH sensor is placed between the detection windows (through which the water vapor from the skin enters) and the silica gel. The underlying principle of the perspiration monitoring device is based on Fick's law of diffusion, which means that water vapor flux from the skin to the silica gel (*i.e.*, transepidermal water loss and perspiration) can be captured by change in humidity at the T/RH sensor. In addition, a baseline subtraction method was adopted to distinguish perspiration and transepidermal water loss.

As shown in the previous report, the developed device can monitor the perspiration at any sites of the body in an easy, wireless manner. However, detailed methods of how to use the device have not been disclosed yet.

In this article, therefore, we would like to show the point-by-point tutorials of how to use the device for perspiration monitoring, by showing the sympathetic activity test with the sympathetic skin response monitoring as an example.

INTRODUCTION:
Human perspiration, generally known as "sweating," is not just a mechanism for thermoregulation[1], but it is also related to certain kinds of diseases. The etiology of abnormal perspiration is broad, including: heatstroke, hyper- or hypothyroidism[2], brain infarction[3], diabetes mellitus[4], dysautonomia[5], menopause (known as "hot flash")[6], cystic fibrosis[7], Parkinson's disease[8], and social anxiety disorder[9]. In light of the number of perspiration-related diseases, it has been considered beneficial to monitor perspiration rates for the early diagnosis or prediction of such diseases (*e.g.,* prevention of heatstroke) in a ubiquitous manner[10].

To date, only a small number of devices for perspiration monitoring have been proposed. In early days, skin conductance and relative humidity were used for indirect indices of the amount of perspiration[11,12]. Most recently, several kinds of flexible, wearable sensors for perspiration monitoring have been proposed[13-19], although they are intended for the analysis of sweat electrolytes rather than the amount or temporal pattern of perspiration. The calculation of water vapor diffusion has been utilized for a more quantitative method of monitoring water exchange from the skin[20-23]. However, this requires (1) the assumption that the outer atmosphere is still and constant[20], (2) enough sensitivity to detect the natural flow of water vapor[21,22], or (3) a coolant (*e.g.*, Peltier device that consumes a substantial amount of electricity) to condense water vapor to liquid[23]; thus, they might be difficult for daily and long-term monitoring. As an alternative, a ventilated chamber method was developed[20,24,25]. In the ventilated chamber method, dry nitrogen or dehumidified air is infiltrated in a small chamber adjacent to the skin from a nitrogen gas tank or a pump, and gas in the water vapor evaporated from the skin is collected. The amount of water vapor from the skin can be

calculated from the difference of the humidity in the outlet and inlet gases. Although this method can estimate the amount of perspiration very precisely, a nitrogen gas tank or a mechanical pump is generally large enough to impede daily monitoring.

To address these drawbacks, we have recently developed a novel device for perspiration monitoring, in which a closed chamber with a desiccant-driven enforced water vapor flow, enabled sensitive and long-term monitoring[26]. This device consists of a cylindrical plastic exterior, temperature/relative humidity (T/RH) sensor with recording microprocessor, and silica gel (Figure 1). In principle, the outer atmosphere should not interfere with the water vapor flow, and a coolant or ventilating chamber is not required. Perspiration profiles can be obtained by solving equations using a spreadsheet software[26]. A previous study has only shown the principle of the developed device and has omitted the detailed method for how to use the device because of space limitations.

The objective of this article, therefore, is to show a detailed method of how to use the developed device for perspiration monitoring, by showing the recording of stress-induced palmar perspiration during the sympathetic activity test as an example.

PROTOCOL:
NOTE: The device, including the method of analysis, is covered by Japanese Unexamined Patent Application Publication No. 2011-169881 and the Japanese Patent No. 5708911.

NOTE: This study, including the protocol of the experiment with human subjects, was approved by the Medical Ethics Committee of Kanazawa University (#553-1).

1. Prerequisites for the perspiration monitoring device
NOTE: Perform these steps only once before the first use.

1.1) Install the universal serial bus (USB)-serial port conversion interface drivers in the computer[27]. If the drivers are already installed, skip this step.

1.2) Connect the USB-serial port conversion interface to the computer via USB cable. Wait for the automatic driver installation if any.

1.3) Set the parameters of the conversion interface and check the ID number of the serial port as follows.

1.3.1) Open the device manager, and select [View] → [Devices by Type].

1.3.2) Locate the "Ports (COM & LPT)" heading, and expand the section to find the heading containing "CP210x USB to UART Bridge" in the list.

1.3.3) Memorize the ID number of the port (*e.g.,* "COM5") in the "CP210x USB to UART Bridge" heading.

1.3.4) Double click the "CP210x USB to UART Bridge," and open the "Port Settings" tab.

1.3.5) Set the parameters as follows: "Bits per second" = 9600, "Data bits" = 8, "Parity" = "None," "Stop bits" = 1, and "Flow control" = "None."

1.3.6) Click the "OK" button to close the window, and close the device manager.

2. Setup of the perspiration monitoring device
NOTE: Set the recording settings as follows before using the device. Repeat these steps for each device if multiple devices are to be used.

2.1) Make sure the battery is **not** inserted in the perspiration monitoring device.

2.2) Plug the white connector of the USB-serial port conversion interface to the receptacle at the top of the perspiration monitoring device.

2.3) Execute the perspiration recording software, followed by setting the device parameters as follows.

2.3.1) Open the "Settings" tab.

2.3.2) Set the "COM" number to the ID number of the serial port (see step 1.3.3).

2.3.3) Click the "Confirm Connection" button. Check if the message "Connected…" appears.

2.3.4) At the "Data File Folder" settings, choose the drive and select the folder where the perspiration data will be saved. To select the subfolder, double click the folder to open.

2.3.5) Set the "Number of holes" to "4."

2.4) Open the "Measure & Rec" tab, and set the "Time Interval" to the desired sample time.

2.5) Insert a battery to the perspiration monitoring device.

2.6) Fill the device with dry silica gel (color should be blue or green if the color indicator is available), and close the lid. If the lid does not completely close, reduce the amount of silica gel.

2.7) Click the "Start Logging Now" button in the perspiration recording software.
NOTE: Because the perspiration recording starts just after the disconnection, the perspiration monitoring device may be left connected until the monitoring is to be started.

3. Setup for the measurement of sympathetic skin response (SSR)
NOTE: These steps are intended to monitor the sympathetic activities as the palmar SSR, and are not necessarily required for perspiration monitoring itself. SSR is a change of skin potential according to the sympathetic arousal stimulation such as upset and concentration[28,29].

3.1) Cleanse the skin where the SSR is to be recorded with an alcohol swab.

3.2) Put the anode, cathode, and grounding electrode on the palm, back of the hand, and wrist, respectively, by means of an electrode paste. Fix the electrodes with medical tape.

3.3) Set the conditions of the instrumentation amplifier by turning the corresponding knobs of the amplifier as follows: Sensitivity = 1 mV/V, High cut filter ("HI CUT") = 3 kHz, and Low cut filter ("LO CUT") = 0.5 Hz.

3.4) Execute the SSR recording software, and start the recording at the sampling rate = 200 Hz by clicking the "Start Measurement" button in the software.

4. Recording of the perspiration
4.1) Put a medical double-sided tape on the bottom of the perspiration monitoring device. When putting a tape, make sure the measurement windows (*i.e.*, four holes at the bottom of the device) are not obstructed.

4.2) Disconnect the perspiration monitoring device from the USB-serial port conversion interface. Immediately after the disconnection, observe the perspiration monitoring begin automatically. Make sure the LED lamp is blinking.

4.3) Remove the release liner of the medical double-sided tape, and put the perspiration monitoring device on the skin where perspiration is to be monitored.

4.4) Wait 10 min for stabilization of water vapor diffusion.
NOTE: Although this process cannot be monitored, the previous study has confirmed that waiting 10 min is sufficient for stable monitoring[26].

4.5) Start the examination (*e.g.*, sympathetic activity test).

4.6.) After the examination, remove the perspiration monitoring device from the skin. To stop the SSR recording, click the "Stop Measurement" button in the SSR recording software, and remove all electrodes from the skin.

5. Perspiration analysis
5.1) Connect the perspiration monitoring device to the USB-serial port conversion interface (as in step 2.2).

5.2) Execute the perspiration monitoring software.

5.3) Open the "Settings" tab, and click the "Confirm Connection" button.

5.4) Open the "Measure & Rec" tab, and click the "Download" button to save the raw data of the device in the specified folder (see step 2.3.4).

5.5) Perform perspiration analysis as follows.

5.5.1) Open the "Analysis" tab, and click the "Read Data File." Observe the "Open File" dialog

NOTE: The software automatically performs perspiration analysis based on the published method[26], and the results will appear on the screen. At the same time, the perspiration data as the comma-separated values format will be saved in the same folder.

REPRESENTATIVE RESULTS:
Using this novel device for perspiration monitoring (Figure 1) and the Fick's law-based calculation, the temporal perspiration profiles can be obtained in an easy, wireless manner.

Figure 2 shows representative data of wireless perspiration monitoring during the sympathetic activity test. In the experiment, the device for the perspiration monitoring, along with the electrodes for the sympathetic skin response (SSR) monitoring, was attached to the subject's palm. For the sympathetic activity test, the subject was requested to sit and perform the following tasks: (1) take a deep inspiration 5 times with 1 min intervals, and (2) do a mental calculation (*e.g.*, continuously subtract 7 from 100, or the "Flash Anzan" in which the participant sums up the number displayed one after the other on a computer screen) to evoke sympathetic activity. The perspiration and the SSR were simultaneously recorded during the stress conditions. As a result of deep inspiration and mental calculation, the sympathetic activity-induced palmar perspiration could be measured using the developed device.

Figure 3 shows representative data of multipoint measurement during daily activities. About one-hour recording of perspiration at the palm (emotional sweating) and the anterior chest (thermal sweating) showed distinct patterns according to the activities.

FIGURE LEGENDS:
Figure 1: The novel device for perspiration monitoring. (A) The exterior of the device containing dry silica gel. (B, C) A "doughnut-shaped" double-sided tape used in this study and its attachment to the device. (D) Attachment of the device to the skin.

Figure 2: Representative recording of perspiration under the sympathetic activity test. (A) Attachment of the perspiration monitoring device with the sympathetic skin response (SSR) electrodes on the palm. (B) As a result of sympathetic activity test, palmar perspiration along with the SSR reaction was observed in response to the sympathetic activities.

Figure 3: Representative multipoint recording of perspiration during the daily activities. The distinct patterns of perspiration according to the different parts of the body (red line, at the palm; blue line, at the anterior chest) were observed during several activities (*e.g.*, going downstairs, driving a car, having a lunch with talking, and shopping).

DISCUSSION:
The aim of this article is to introduce the use of a novel, wireless perspiration monitoring device. Owing to the recent progress of engineering, more accurate, easy-to-handle methods for temporal perspiration monitoring have been proposed; the ventilated chamber method[24,25] and the vapor pressure diffusion method[23] are representative examples. However, the ventilated chamber method requires the use of dry nitrogen or a pump with desiccant to produce a dry atmosphere, and thus, the exterior tends to be large. The vapor

pressure diffusion method can be adopted in a small exterior, although the open chamber system can be largely influenced by the outer atmosphere, and the closed chamber system has a problem with either water vapor saturation (the chamber is filled with a saturated water vapor) or considerable power consumption of a water vapor condenser (*e.g.*, Peltier device).

To address the situation, we have recently developed a novel wireless device for perspiration monitoring[26]. In the device, the water vapor from the skin can be captured by a desiccant, enabling a constant but natural flow. In the course of the water vapor traveling, the T/RH sensor captures the flow of the water vapor as a change in the temperature and relative humidity. By calculating such changes under Fick's law, we could estimate the temporal changes of perspiration amount ($mg/cm^2/min$) with <5% error, relative to the conventional ventilated chamber method[26]. The developed device can be used, for example, to monitor mental stress (Figure 2), thermal sweating, and ultimately the perspiration-related dysregulation in an easier manner.

The developed device is easy to handle, therefore there are few points to consider. However, the perspiration measurement would fail if the silica gel is not dry. Before the experiment, therefore, the examiner should make sure that the silica gel is completely dry (*i.e.*, the color is blue). A previous study has estimated the maximum duration of measurement being longer than 4 hr without changing the silica gel[26]. To renature silica gel, dry it in a drying oven until the color turns blue. A conventional microwave oven is also useful. The perspiration measurement would also fail if the attachment of the device is not sufficient. We recommend the usage of a "doughnut-shaped" double-sided tape.

Nonetheless, the developed device has a limitation. Because of the delicate calculation and the limit of desiccant absorptivity, the error of the amount of perspiration should be considered, especially at the higher level of perspiration. Although we have confirmed the error rate as being <5% relative to the conventional method[26], the absolute value of the calculated perspiration value should be handled with caution.

Owing to the small, simple, and wireless design of the device, the multipoint perspiration monitoring under unrestricted conditions can be possible. As shown in Figure 3, the different temporal profiles of perspiration can be detected on the different positions of the skin (*i.e.*, palm and anterior chest) in daily life conditions (*e.g.*, talking, eating foods, driving a car, shopping, *etc.*). The device, therefore, might be utilized for the simultaneous detection of abnormal sweating derived both from the mental and thermal regulatory system malfunction. For example, the dysregulation of the peripheral nervous system in diabetes might be detected if the unbalanced sweating between the left and right soles were to be detected. We are now planning an observational study of perspiration profiles in hospitalized patients as a clinical experiment.

Unexamined Patent Application Publication No. 2011-169881 and the Japanese Patent No. 5708911.

DISCLOSURES:
The author Fukuoka Masakazu is the president of the Rousette Strategy Inc., where the developed device was assembled. No financial support was received from either Fukuoka Masakazu or the Rousette Strategy Inc.

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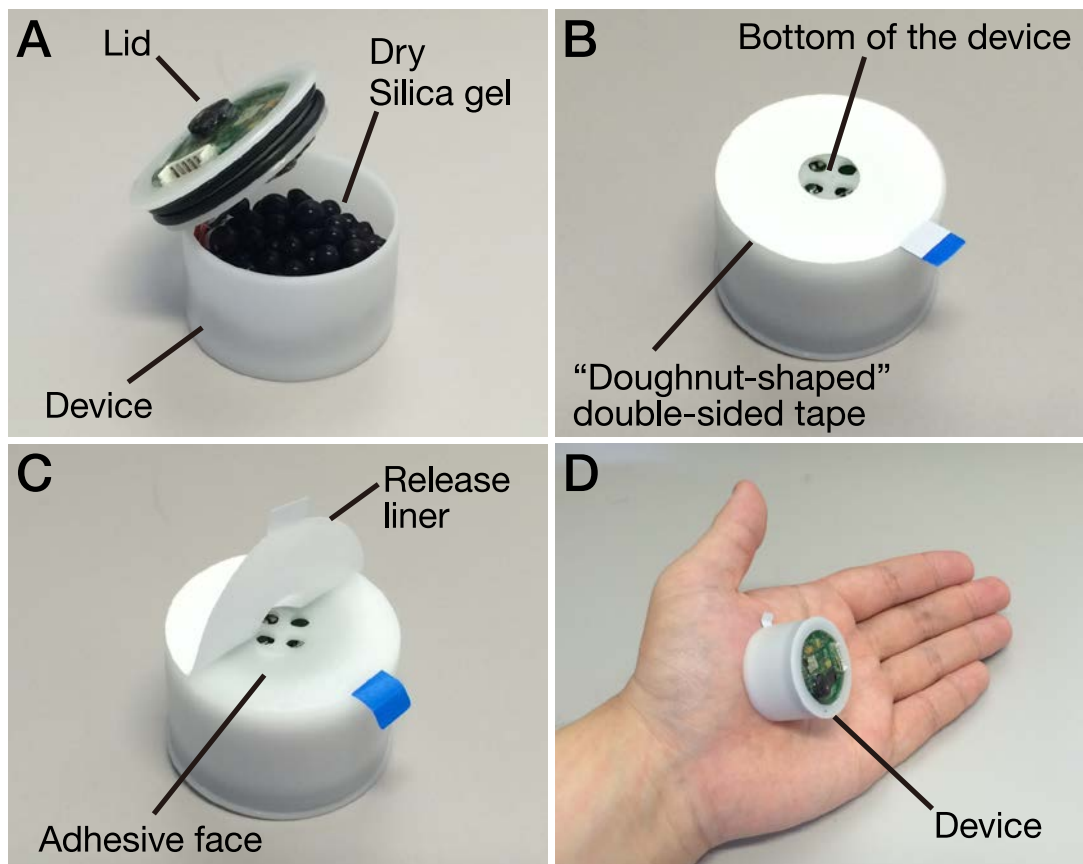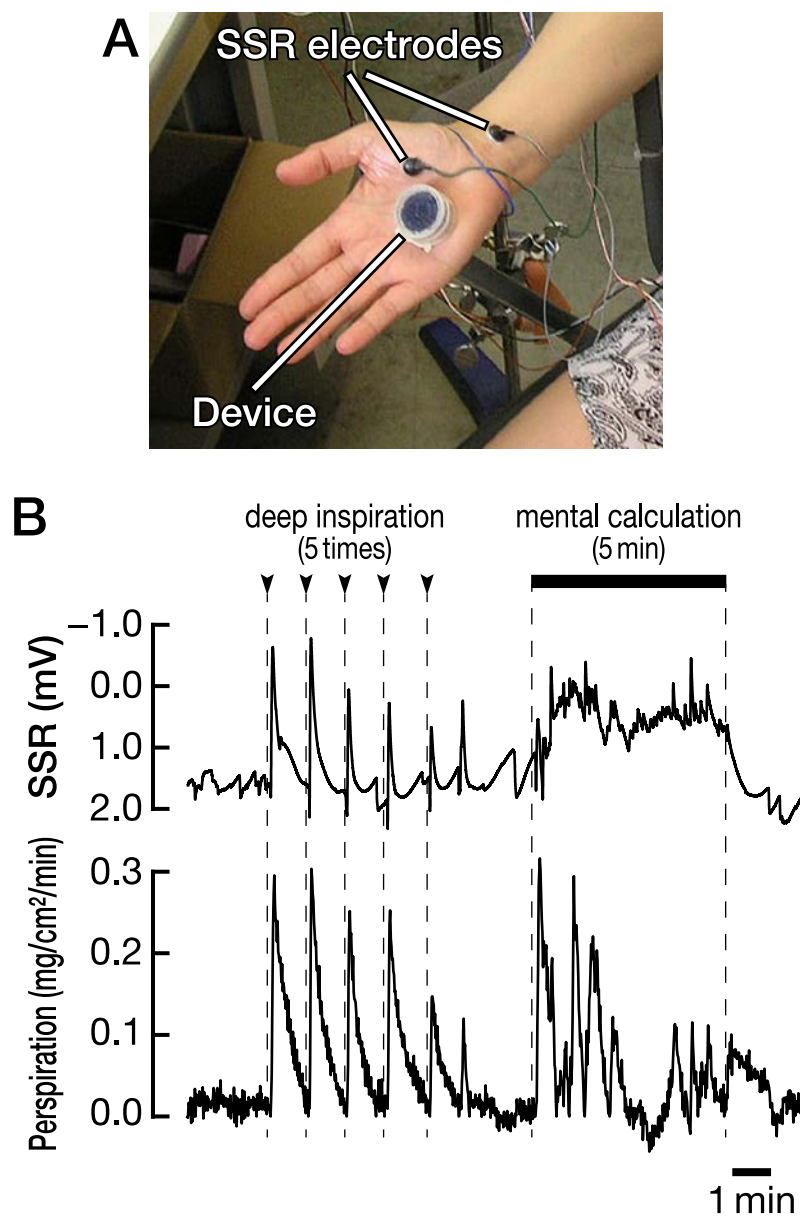
Fig. 1

A — Lid, Dry Silica gel, Device

B — Bottom of the device, "Doughnut-shaped" double-sided tape

C — Release liner, Adhesive face

D — Device

Fig. 2

A

SSR electrodes

Device

B

deep inspiration
(5 times)

mental calculation
(5 min)

SSR (mV)

−1.0
0.0
1.0
2.0

Perspiration (mg/cm²/min)

0.3
0.2
0.1
0.0

1 min

Fig. 3