Analytical and Bioanalytical Chemistry (2021) 413:1883–1891
https://doi.org/10.1007/s00216-021-03156-3

RESEARCH PAPER

A fully screen-printed potentiometric chloride ion sensor employing a hydrogel-based touchpad for simple and non-invasive daily electrolyte analysis

Yusuke Ichimura[1] · Takumi Kuritsubo[1] · Kuniaki Nagamine[1,2] · Ayako Nomura[2] · Isao Shitanda[2,3] · Shizuo Tokito[1,2]

Received: 5 March 2020 / Revised: 23 December 2020 / Accepted: 5 January 2021 / Published online: 22 January 2021

Abstract

This is the first report demonstrating proof of concept for the passive, non-invasive extraction and in situ potentiometric detection of human sweat chloride ions (Cl^- ions) using a stable printed planar liquid-junction reference electrode-integrated hydrogel-based touch-sensor pad without activities such as exercise to induce perspiration, environmental temperature control, or requiring cholinergic drug administration. The sensor pad was composed entirely of a screen-printed bare Ag/AgCl-based chloride ion-selective electrode and a planar liquid-junction Ag/AgCl reference electrode, which were fully covered by an agarose hydrogel in phosphate-buffered saline (PBS). When human skin contacted the hydrogel pad, sweat Cl^- ions were continuously extracted into the gel, followed by in situ potentiometric detection. The planar liquid-junction Ag/AgCl reference electrode had a polymer-based KCl-saturated inner electrolyte layer to stabilize the potential of the Ag/AgCl electrode even with a substantial change in the chloride ion concentration in the hydrogel pad. We expect this fully screen-printed sensor to achieve the low-cost passive and non-invasive daily monitoring of human Cl^- ions in sweat in the future.

Keywords Biosensor · Sweat analysis · Printed electronics · Hydrogel · Healthcare

Introduction

To realize future non-invasive preventive medicine and healthcare, non-invasive diagnostics are expected to be alternatives to conventional blood sampling-based diagnostics. Biological fluids, such as tears [1, 2], saliva [3, 4], urine [5, 6], and sweat [7], are used to non-invasively access various biomarkers partitioned from the blood. Sweat secreted from the eccrine gland is readily collected from the surface of human skin, and its composition reflects some physiological information [8, 9]. For example, chloride ions (Cl^- ions), which are one of the primary electrolytes in human sweat (concentration range of 10–100 mM [10]), have been considered as a biomarker for cystic fibrosis (CF) [11, 12] and dehydration [13, 14]. For the diagnosis of CF, values over 60 mmol/L are considered to be diagnostic of classic CF disease. Recent guidelines recognize an intermediate level (in the USA: 30–59 mmol/L for infants, 40–59 mmol/L for individuals N6 months old; in Europe: 30–60 mmol/L for all age groups) that requires further testing and diagnostic evaluation. Healthy individuals have lower than 39 mM (29 mM for individuals N6 months of age) [15]. Therefore, sweat components are expected to be favorable biomarkers for future preventive medicine and healthcare.

Although sweat glands are distributed throughout the body (more than 100 glands per cm^2 of skin), their perspiration rate is too slow (in the order of nL/min/gland); hence, it is challenging to collect enough sweat for subsequent analysis [10, 16]. Microfluidic-based wearable sweat sensors are one of the promising ways to effectively manage small amounts of sweat from the skin [17–24]. However, this passive sweat collection method requires physical activity to induce perspiration, such as intense exercise [25], environmental temperature control

✉ Kuniaki Nagamine
nagamine@yz.yamagata-u.ac.jp

✉ Shizuo Tokito
tokito@yz.yamagata-u.ac.jp

[1] Graduate School of Organic Materials Science, Yamagata University, 4-3-16 Jonan, Yonezawa, Yamagata 992-8510, Japan

[2] Research Center of Organic Electronics (ROEL), Yamagata University, 4-3-16 Jonan, Yonezawa, Yamagata 992-8510, Japan

[3] Department of Pure and Applied Chemistry, Faculty of Science and Technology, Tokyo University of Science, Noda, Chiba 278-8510, Japan

[18, 26], and the iontophoretic administration of perspiration-inducible drugs [17, 27, 28]. These active processes for inducing sweating are not acceptable for daily use. Therefore, to overcome this issue, we established a new sweat sensor for the first time, which requires to only touch the surface of the agarose hydrogel that thoroughly covers the sensor electrodes to extract and in situ detect sweat components [29, 30]. This new type of simple sensor is receiving attention in the sweat analysis field [31] and is expected to enable the daily monitoring of human sweat biomarkers anytime and anywhere. Additionally, to widely spread this sensor at a low cost in the future, we are considering the mass-production of this sensor using advanced printing methods [32–34].

Previously, our hydrogel-based sweat sensor used a bare Ag/AgCl electrode as a pseudo reference electrode for easy fabrication [29, 30]. However, the potential of the bare Ag/AgCl pseudo reference electrode tends to change depending on the sweat Cl^- ion concentration extracted in the agarose hydrogel. This potential drift was suppressed using phosphate-buffered saline containing a high level of chloride (140 mM) as a sweat extraction solution in the gel in a previous report. In this case, the change in the electric potential for an increase in the Cl^- ion concentration by 10 mM was calculated to be a few mV using the Nernst equation. However, it may cause significant noise when measuring small potentiometric signals derived from the low concentration of sweat biomarkers. In particular, for the measurement of Cl^- ions in sweat, the potentials of the Cl^- ion-selective electrode and the bare Ag/AgCl pseudo reference electrodes can change together in the same direction, resulting in no potentiometric response. For stable measurements, a planar reference electrode with high potential stability [35–38] should be employed in the hydrogel-based sweat sensors.

In this study, we integrated a planar liquid-junction Ag/AgCl reference electrode into a hydrogel-based sweat sensor for the stable monitoring of human sweat Cl^- ions. The sensor was composed of a fully screen-printed bare Ag/AgCl-based Cl^- ion-selective electrode and a planar liquid-junction Ag/AgCl reference electrode. The planar liquid-junction Ag/AgCl reference electrode was composed of an Ag/AgCl electrode covered by a KCl-saturated polymer-based internal electrolyte layer, ionically connected to the external agarose hydrogel pad via a polyurethane-based liquid junction. To achieve the potential long-term stability of the reference electrode by shortening the setup time defined below, we studied the effect of mixing hydrophilic polyethylene glycol with polyurethane to tune the ionic conductance of the liquid junction. To our knowledge, this is the first report demonstrating the passive extraction and in situ potentiometric detection of Cl^- ions from human sweat, without activities, such as intense exercise, environmental temperature control, or cholinergic drug administration.

Materials and methods

Quantification of chloride ions in the extracted human sweat using a colorimetric method

The index finger of the subject was washed with running 1 vol% ethanol-water for 15 s. Afterward, 30 μL of phosphate-buffered saline (PBS, KH_2PO_4: 1.47 μM, Na_2HPO_4: 8.1 μM, KCl: 2.68 μM, NaCl: 137 μM, pH 7.4) was added to a 0.6 mL microcentrifuge tube (ASONE), followed by putting it on the subject's finger so that the skin was in contact with PBS for 10 min. After collecting the PBS from the tube, the concentration of the Cl^- ions was quantified using a commercially available colorimetric chloride assay kit (QuantiChrom™ Chloride Assay Kit, DICL-250).

Fabrication of the electrodes using a screen-printing method

Figure 1a illustrates the structure of the planar liquid-junction Ag/AgCl reference electrode fabricated using a screen-printing technique. A silver paste (TYPE. FA-323, FUJIKURA KASEI Co., Ltd.) was printed as a lead pattern on a polyethylene naphthalate (PEN, thickness: 125 μm) substrate and baked for 30 min at 150 °C to evaporate the silver paste solvent. Next, an Ag/AgCl paste (Code. No. C2130809D5, Sun Chemical) was printed on the active area of the silver-lead pattern and baked for 30 min at 120 °C. An internal electrolyte paste composed of 50 wt% of polyvinylpyrrolidone K30 (PVP, an average molecular weight of 40,000, Tokyo Chemical Industry Co., Ltd.) in a saturated KCl aqueous solution was screen-printed over the Ag/AgCl active layer and baked at 25 °C overnight to prevent the generation of bubbles derived from the evaporated solvent. Next, a liquid-junction ink composed of 10 wt% polyurethane (PU, S90A110, BASF Co., Ltd.) in 1-Methyl-2-pyrrolidinone (NMP) (133–15115, FUJIFILM Wako Pure Chemical Corporation) was screen-printed on one end of the electrolyte layer and baked at 25 °C for 3 h. Finally, the entire structure, except for the tip of the liquid junction, was covered by a screen-printed UV-curable insulator paste (JELCON, No. IN-15M, Jujo Chemical Co., Ltd.) and irradiated with 302 nm UV for 20 min. Figure 1b shows an image of the resulting reference electrode captured by a laser microscope (LEXT OLS4000, Olympus, Japan).

For a Cl^- ion-selective electrode, a bare Ag/AgCl electrode was used as the active area. The silver line pattern was printed in the vicinity of the planar liquid-junction Ag/AgCl reference electrode pattern, and afterward, an Ag/AgCl layer was printed at the edge of the silver-based line pattern under the same conditions as described above.

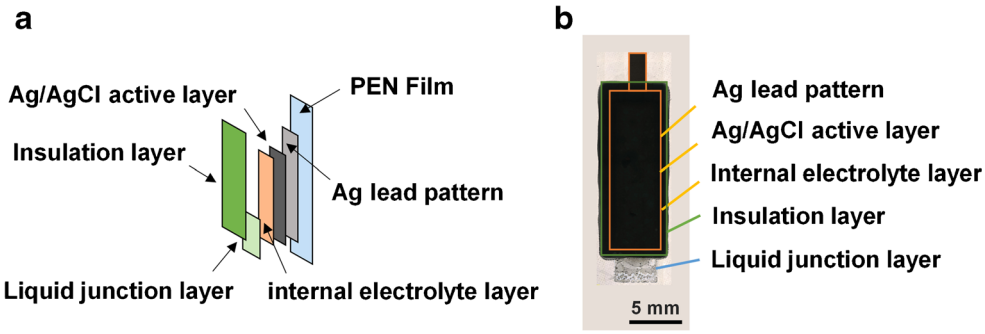

Fig. 1 Structure (**a**) and optical microscope image (**b**) of the screen-printed planar liquid-junction Ag/AgCl reference electrode

Electrochemical characterization of the printed electrodes

The potentiometric response of a bare Ag/AgCl-based Cl^- ion-selective electrode was measured in PBS measurement solution using a commercially available Ag/AgCl reference electrode (RE-1B, BAS Inc.). Concentrated KCl in PBS was periodically added to the PBS measurement solution and continuously stirred at 200 rpm while monitoring the potential difference between the working and reference electrodes using an electrochemical analyzer (ALS model 602E, BAS Inc.) operated in the open-circuit potential mode (input impedance: 1×10^{12} Ω).

To measure the stability of the potential of the fabricated reference electrode, the planar liquid-junction Ag/AgCl electrode was immersed in 0.1 mM KCl measurement solution, and the potential difference against a commercially available Ag/AgCl reference electrode (RE-1B, BAS Inc.) was monitored while periodically adding concentrated KCl aqueous solution into the measurement solution.

Potentiometric measurements of externally extracted sweat Cl^- ions using the sensing device

The sensing device was composed of a bare Ag/AgCl-based Cl^- ion-selective electrode and a planar liquid-junction Ag/AgCl reference electrode printed on a PEN substrate. Twenty microliters of the PBS measurement solution were dropped on the sensing device to fully cover the tips of the bare Ag/AgCl-based Cl^- ion-selective electrode and planar liquid-junction Ag/AgCl reference electrode. After stabilizing the potential difference between these electrodes, another 10 μL of the PBS containing sweat Cl^- ions previously extracted from the subject's finger for 10 min was added to 20 μL of the PBS measurement solution while monitoring the potentiometric response of the sensing device. For comparison, the same protocol was conducted using a pair of bare Ag/AgCl electrodes printed on a PEN substrate.

Fabrication and characterization of the agarose hydrogel-covered sweat Cl^- ion extraction and in situ detection device

A silicone rubber sheet (2 mm thick, ASONE) with a through-hole (10 mm × 10 mm square) was attached to the sensing device composed of the bare Ag/AgCl-based Cl^- ion-selective electrode and planar liquid-junction Ag/AgCl reference electrode printed on the PEN substrate to expose the tips of both electrodes via the through-hole. A 4 wt% low-melting-point agarose gel (Nakarai Tesque) prepolymer solution in PBS was dropped into the through-hole of the silicone sheet and incubated at room temperature for 10 min to induce gelation. The index finger of the subject was washed using running 1 vol% ethanol-water for 15 s immediately before the experiment. After stabilizing the potentiometric response of the sensing device, the side of the subject's index finger was placed on the surface of the agarose gel for 5 min; afterward, it was removed from the gel. During this process, the potential difference was continuously monitored using an electrochemical analyzer operated in open-circuit potential mode.

Results and discussion

Before fabricating the sensing device, we used the previous sweat extraction protocol [29, 30] and a commercially available colorimetric assay kit to quantify the concentration of human sweat Cl^- ions extracted in PBS (pH 7.4). This assay kit can be used for raw biological samples without any pretreatment. We confirmed the linear detection range of the colorimetric assay kit was from 0.2 to 10 mM. The standard deviation in this linear detection range was 0.007 ± 0.004 mM ($n = 3$), suggesting the quantitativeness of this assay kit. To eliminate individual differences, only one healthy subject was tested in this study. The extraction of sweat Cl^- ions from the subject's index finger for 10 min once every 2 h was repeated three times to evaluate its circadian rhythm. The calibration curve prepared by following the product's protocol was used to calculate the concentration of the extracted sweat Cl^- ions (3.7 ± 3.2 mM ($n = 3$)). The variations of the

extracted human sweat Cl^- ion concentration was not due to uncertainties in the assay kit, but from the natural variations of Cl^- ion concentration in the extracted human sweat. In this study, we were able to confirm that the potential of the Ag/AgCl reference electrode should be stable even when Cl^- ion concentration in the PBS extraction solution changed from 140 μM (original concentration in PBS) to 10 mM (the value was set to more than the maximum value of the extracted sweat Cl^- ion concentration).

Figure 1a illustrates the structure of the screen-printed planar liquid-junction Ag/AgCl reference electrode composed of a silver-lead pattern, an Ag/AgCl active layer, an internal electrolyte layer, a liquid junction, and an insulation layer on a PEN film. The Ag/AgCl active layer was covered by the KCl-saturated PVP-based internal electrolyte layer to maintain the concentration of the Cl^- ions around the Ag/AgCl active layer constant. The polyurethane-based liquid-junction layer was printed on one end of the internal electrolyte layer to form an ionic connection between the internal electrolyte layer and the external measurement solution. The entire structure, except for the tip of the liquid junction, was covered by a UV-curable insulation layer. In this configuration, when the tip of the planar liquid-junction Ag/AgCl reference electrode was immersed in the measurement solution, the measurement solution gradually entered the internal electrolyte layer through the polymer-based liquid junction, followed by swelling of the inner electrolyte layer. Thus, the Ag/AgCl electrode was ionically connected to the external measurement solution while maintaining its internal Cl^- ion concentration at a saturation level. We defined the time required to form an ionic connection between the Ag/AgCl active layer and the external measurement solution as the "setup time," and the time where the potential drift of the printed reference electrode was less than 10 mV as the "operation time."

Figure 2 shows the inherent stability of the screen-printed planar liquid-junction Ag/AgCl electrode against a commercially available Ag/AgCl reference electrode. These two electrodes were immersed in 100 mM KCl aqueous solution stirred at 200 rpm at room temperature, and the potential difference between them was monitored using a voltmeter. The operation time in the insets in Fig. 2 is indicated by the gray-colored area. The areas before and after the gray color represent the setup time and broken time, respectively. In this case, the broken time could be confirmed from the shift in the detected potential difference by approximately +50 mV from the stable value, where the internal saturated KCl was completely exchanged by the external 100 mM KCl solution. The printed planar Ag/AgCl electrode with a polyurethane-based liquid junction showed a setup time of 50 min, and the operation time was over 40 h (Fig. 2b). When hydrophilic polyethylene

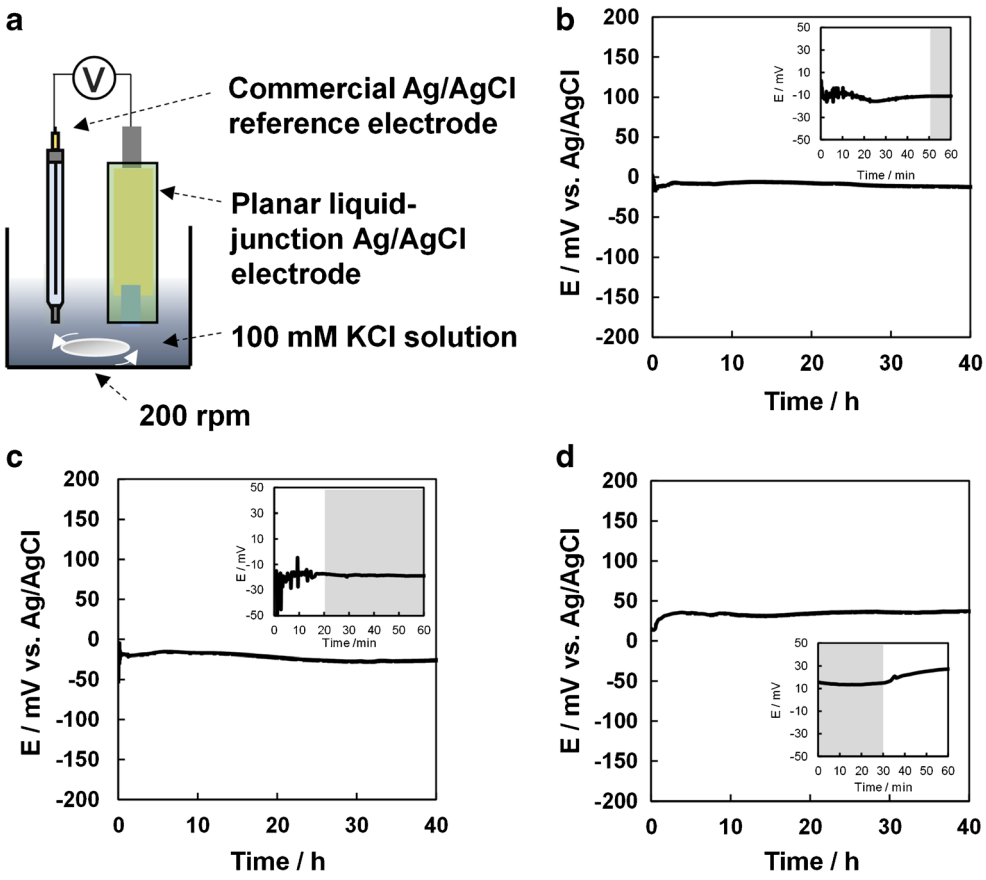

Fig. 2 **a** Setup for the evaluation of the potential stability of the planar liquid-junction Ag/AgCl electrode. **b**–**d** Potential changes of the planar liquid-junction Ag/AgCl electrode when PEG was mixed in the liquid-junction ink in the concentration of **b** 0 wt%, **c** 8.8 wt%, and **d** 32.8 wt%. Insets of each graph show the magnified data at the time from 0 to 1 h

glycol (PEG, Mw: 1500) was mixed in the polyurethane-based liquid-junction ink (concentration of PEG: 8.8 wt%), the setup time decreased to 20 min (Fig. 2c). The further addition of PEG to the liquid-junction ink (concentration of PEG: 32.8 wt%) decreased the setup time to 1 min, while the operation time was only 30 min (Fig. 2d). These results suggest that the hydrophilic PEG polymer might act as an ion-conductive pathway in a hydrophobic polyurethane-based liquid junction [39, 40]. Therefore, the setup time and operation time can be arbitrarily tuned by adjusting the amount of PEG mixed in the liquid-junction ink. In the following study, a liquid-junction ink containing 8.8 wt% of PEG was employed as a model to secure a sufficient operation time for the sensor electrode. In a future study, the influence of the molecular weight of PEG on the setup time and operation time, and the three-dimensional morphology of the liquid-junction layer should be studied to understand in detail the ion conduction mechanism and optimize the setup and operation time for actual use.

Afterward, the inherent stability of the planar liquid-junction Ag/AgCl electrode in relation to the changes in the external Cl^- ion concentration was evaluated using the setup shown in Fig. 3a. For comparison, the potential stabilities of a printed bare Ag/AgCl electrode were evaluated. When stepwise changes to the external Cl^- ion concentration were made from 0.1 mM to 10 mM, the potential of the planar liquid-junction Ag/AgCl electrode was virtually constant. In contrast, only approximately 20 mV of the potential change was observed at the concentration step from 10 to 100 mM (red line). However, the potential of a bare Ag/AgCl electrode, which was our previous pseudo reference electrode [29], showed a stepwise change, and the total potential change was approximately 160 mV (black line). These results suggest that the potential of the planar liquid-junction Ag/AgCl electrode was quite stable when the concentration range of the extracted sweat Cl^- ions was from 0.1 to 10 mM, suggesting the suitability of this material as a reference electrode in a hydrogel-based sweat sensor.

Cyclic voltammetry (CV) measurements were performed in 10 mM ferrocyanide in 0.1 M KCl aqueous solution using the planar liquid-junction Ag/AgCl electrode and a commercially available Ag/AgCl reference electrode. Fig. S1 (see Supplementary information (ESM)) shows the CV curves obtained using a three-electrode configuration at a scan rate of 0.01 mV s^{-1}. Similar CV curve forms were obtained for both reference electrodes, suggesting the stability of the printed reference electrode. Conversely, a small shift in the redox potential was observed; it was probably due to the different Cl^- concentration of the internal electrolyte (the commercial Ag/AgCl reference electrode contains 3 M Cl^- as an internal electrolyte), the intrinsic potential difference between the printed and commercial Ag/AgCl electrode, or a potential drop at the liquid junction.

Afterward, the planar liquid-junction Ag/AgCl electrode was used as a reference electrode to detect the sweat Cl^- ions previously extracted into PBS. The bare Ag/AgCl electrode was used as a Cl^- ion-selective electrode because it shows a potentiometric response against Cl^- ions, as shown in Fig. 3b and Fig. S2 (see ESM), according to the following redox reaction.

$$\mathrm{AgCl} + \mathrm{e}^- = \mathrm{Ag} + \mathrm{Cl}^-,$$
$$E = E^0 - \frac{RT}{F}\ln[Cl^-],$$

Fig. S2(a) (see ESM) shows the potentiometric response of the bare Ag/AgCl-based Cl^- ion-selective electrode in the agarose gel in PBS. To the PBS solution, which originally contained 0.14 mM of Cl^-, 0.1, 1, 10, and 100 mM of NaCl was added; afterward, the mixtures were used to prepare the agarose gel (10 mm × 10 mm × 1 mm). To measure the potentiometric response, the Ag/AgCl-based sensor electrode and

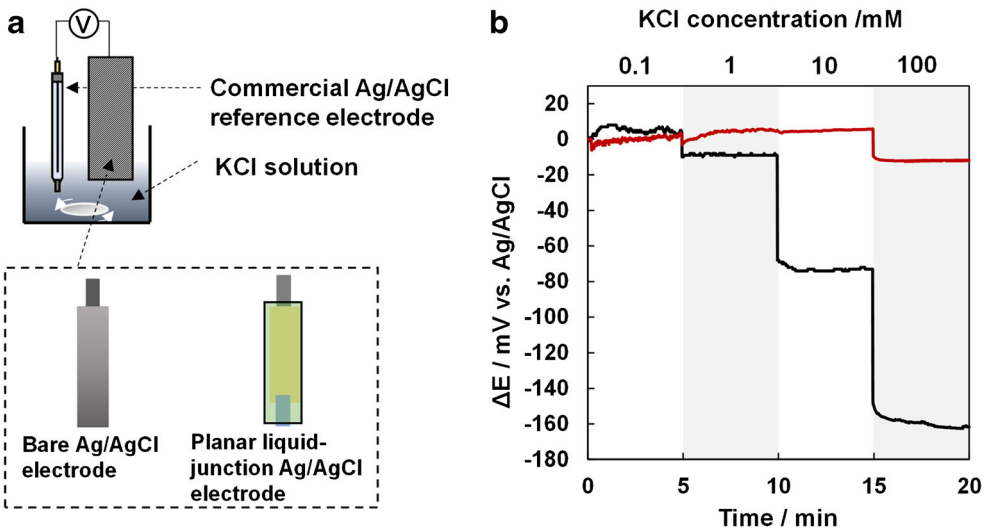

Fig. 3 **a** Setup for the evaluation of the potential stability of the planar liquid-junction Ag/AgCl electrode after changes in external Cl^- ion concentration. **b** The potential changes of the planar liquid-junction (red line) and bare Ag/AgCl electrode (black line) vs. those of a commercially available Ag/AgCl reference electrode monitored with stepwise changes in the concentration of external Cl^- ions

planar liquid-junction Ag/AgCl reference electrode were sequentially covered in the PBS by the hydrogels containing 0.14, 0.24, 1.1, 10.1, and 100.1 mM of Cl^- ions. Fig. S2(b) (see ESM) shows the average ΔE during 100 s of each measurement vs. the concentration of Cl^- ions. The slope calculated from the calibration curve was − 55.1 ± 2.6 mV/dec (n = 4). The limit of detection determined in PBS from 3 SD/slope was 0.11 mM. A bare Ag/AgCl-based Cl^- ion-selective electrode had been utilized to quantify sweat Cl^- in many previous studies. For example, J. Gonzalo-Ruiz et al. reported the good quantification capability of bare Ag/AgCl-based Cl^- ion-selective electrodes for sweat Cl^- analysis compared to that of the sweat Cl^- analyzer traditionally used in hospitals [41]. Representative anionic interferences in the human sweat are as follows: HCO_3^- (0.5–5 mM) [7], phosphate (57 ± 33 μM) [42], SO_4^{2-} (~ 0.1 mM) [43], and NO_3^- (several ten μM) [44]. The selectivity coefficients of the Ag/AgCl-based Cl^- ion sensor against these interferences ($K_{Cl,j}^{pot}$) were evaluated using the separate solution method. $K_{Cl,j}^{pot}$ for each interference were − 1.66 (HCO_3^-), − 1.70 (PO_4^{2-}), − 1.69 (SO_4^{2-}), and − 1.53 (NO_3^-). Fig. S3 (see ESM) shows the potentiometric response of the Ag/AgCl-based Cl^- ion sensor against 1 mM HCO_3^-, 0.05 mM PO_4^{2-}, 0.1 mM SO_4^{2-}, and 0.05 mM NO_3^-, suggesting negligible potentiometric response against these representative anionic interferences.

Figure 4a shows the setup for the detection of the extracted human sweat Cl^- ion. The bare Ag/AgCl-based Cl^- ion-selective electrode and planar liquid-junction Ag/AgCl reference electrode were screen-printed on the same PEN film, as shown in Fig. 4b. Twenty microliters of PBS measurement solution were previously applied to these electrodes, and the potential difference between them was monitored until a stable potentiometric signal was achieved. Next, 10 μL of PBS containing extracted sweat Cl^- ions was added to the PBS measurement solution while monitoring the change in potential difference. The potential difference decreased by approximately 30 mV after adding the PBS containing extracted sweat Cl^- ions, while almost no potentiometric response was observed for the PBS without sweat (Fig. 4c). Conversely, when a pair of bare Ag/AgCl electrodes was used as the working and reference electrodes, no potentiometric response was observed for the PBS with and without extracted sweat Cl^- ions (Fig. 4d). This is because both of the bare Ag/AgCl electrodes exhibited the same negative potential shift against Cl^- ions according to the above Nernst equation. These results suggest the stability of the planar liquid-junction Ag/AgCl reference electrode in the PBS-based sweat extraction solution. The concentration of Cl^- ions extracted in the PBS was calculated to be 1.55 mM from the following equation:

$$\Delta E = -\frac{RT}{F}\ln\frac{[Cl^-]_{after}}{[Cl^-]_{before}},$$

where $[Cl^-]_{before}$ is the concentration of Cl^- in PBS measurement solution (0.14 mM), $[Cl^-]_{after}$ is the final Cl^- concentration after adding the sweat-extracted PBS, ΔE is the potential change upon the addition of the sweat-extracted PBS, and RT/F is the slope of the potentiometric response of the bare Ag/AgCl electrode calculated from ESM Fig. S2 (− 52.8 ± 2.5 mV/dec (n = 4)). This value is within the extracted

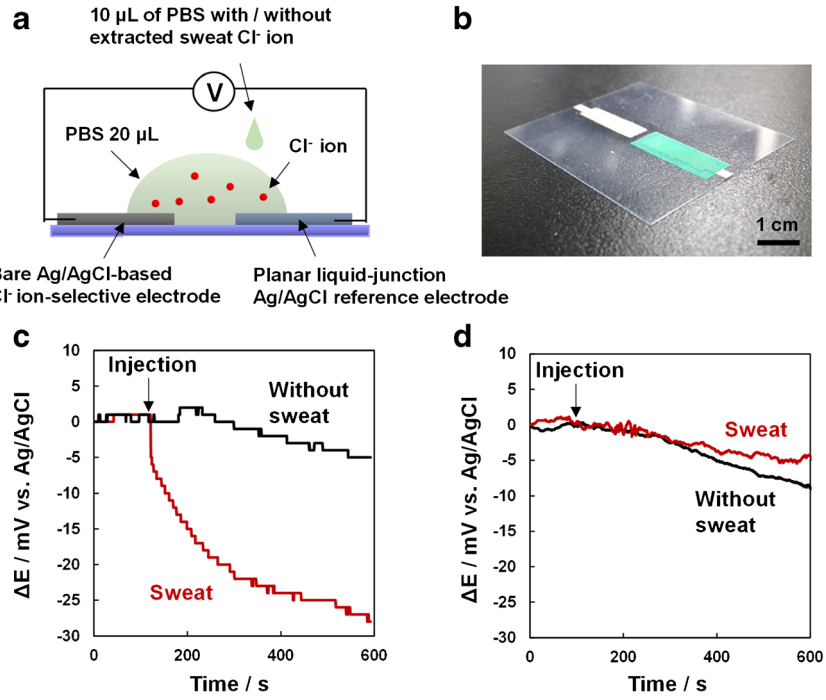

Fig. 4 **a** Schematics of the setup for the detection of human sweat Cl^- ions previously extracted into the PBS using the screen-printed Cl^- ion sensor. **b** A photograph of the fabricated Cl^- ion sensor. **c** The potentiometric response of the fabricated Cl^- ion sensor upon the addition of PBS with and without the extracted sweat Cl^- ions. **d** The potentiometric response of the sensor composed of a pair of bare Ag/AgCl electrodes after the addition of PBS with and without the extracted sweat Cl^- ions

sweat Cl^- concentration range quantified using a commercially available colorimetric assay kit. In this case, as the volume of the measurement solution was quite small (20 μL), the influence of Cl^- ions leaked from the internal electrolyte layer of the planar Ag/AgCl reference electrode via its liquid junction cannot be ignored. We quantified the leakage of Cl^- ions from the printed reference electrode. The printed reference electrode was immersed in 10 mL of PBS, and 20 μL of PBS was sampled periodically to quantify the Cl^- ion concentration using a colorimetric Cl^- ion assay kit. The rate of Cl^- ion leakage from the planar Ag/AgCl reference electrode was 0.03 μmol/h, and it was 1.65 μmol/h for the commercially available Ag/AgCl electrode. From this value, the change in Cl^- ion concentration after 10 min of operation in the 30 μL of measurement solution was 0.17 mM, which is small enough to be ignored.

Finally, we demonstrated the direct extraction and in situ detection of the subject's sweat Cl^- ions using a hydrogel-covered printed Cl^- sensing device. Figure 5a and b show the structure and photograph of the device. The tips of the bare Ag/AgCl-based Cl^- ion-selective electrode and planar liquid-junction Ag/AgCl reference electrode printed on the PEN film was covered with 4 wt% agarose gel in PBS (10 mm × 10 mm, 2 mm in thickness) surrounded by a silicon rubber sheet. After stabilizing the potentiometric response of the device, the subject's finger was washed with running 1 vol% ethanol-water before placing it on the surface of the agarose gel for 5 min (Fig. 5b), and afterward, it was released from the gel. During this process, the potential difference was continuously monitored using a voltmeter (Fig. 5c). The potential began to change 2 min after putting the finger on the gel, and approximately − 20 mV of steady potentiometric response was observed after 20 min. The concentration of Cl^- ions in the gel calculated from − 20 mV of a potentiometric signal using the Nernst equation above was 0.33 mM, while the concentration of Cl^- ions leaked from the reference electrode was calculated to be 0.05 mM. To our knowledge, this is the first report on non-invasive and straightforward sweat Cl^- ion extraction and in situ detection using a portable sensing system without any perspiration assistance, such as exercise, environmental temperature control, or a cholinergic agonist. The design concept of our present sweat sensor is considerably different from that of previous passive sweat sensors employing a microfluidic system or an iontophoretic drug administration system. We consider that this new type of sweat sensor is disposable and useful for the periodic monitoring of sweat analytes. People can extract and detect sweat components from their skin by touching the agarose hydrogel anytime and anywhere. This process can be repeated every day using a disposable sweat sensor chip in the future.

Conversely, the quantitativeness of the present device is still immature. As shown in Fig. 5c, a small potential drift was observed after 20 min, while the potentiometric response of the Ag/AgCl-based sensor in the agarose gel was quite fast and stable over a short measurement time, as shown in ESM Fig. S2(a). Fig. S4 (see ESM) shows another long-term potentiometric response of the sensor when the subject's finger touched the agarose gel for 5 min (gray area). When the surface of the agarose hydrogel was covered by mineral oil at the time of 20 min, the potential drift was slightly suppressed while the potential drift still continued. This result suggested that increased Cl^- ion concentration due to the evaporation of water in the hydrogel would be one of the reasons for the

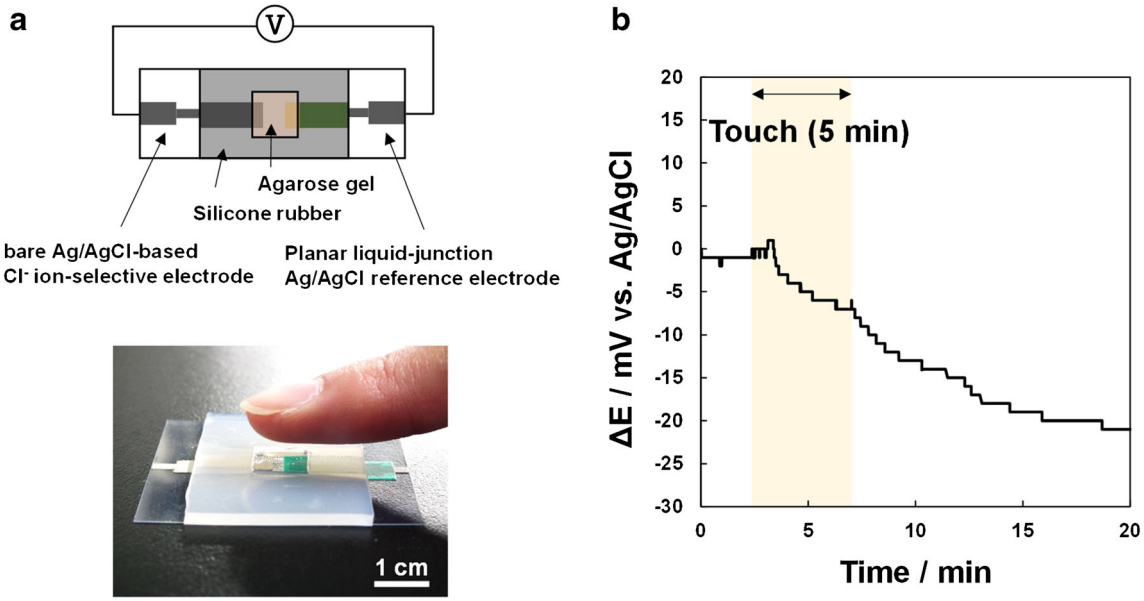

Fig. 5 **a**, **b** Schematic view of the hydrogel-covered sweat Cl^- ion sensing device (**a**) and its photograph (**b**). **c** The potentiometric response of the sensor when the ball of the subject's finger contacts the agarose gel surface for 5 min

potential drift. Therefore, the Cl^- ion concentration was tentatively calculated from ΔE at a time of 20 min in this study. Another factor affecting the sensor performance is the change in hydrogel temperature when the subject touches the hydrogel. An agarose hydrogel (2 mm thick) was placed on a commercially available flat temperature sensor built in a pH sensor (S2K922, ISFETCOM Co., Ltd.), and afterward, the hydrogel was touched by the subject's finger for 5 min to monitor the temperature change. The average ± standard deviation of the temperature change was 3.0 ± 1.7 °C ($n = 3$). This temperature change could cause a negligible change in the slope by 1.01-times (− 55.1 mV/dec to − 55.7 mV/dec) calculated from the Nernst equation. Further, the bare Ag/AgCl-based Cl^- ion-selective electrode has an issue in terms of quantitativeness. The standard deviation for each point in the calibration curve (ESM Fig. S2(b)) was still large, suggesting "semi-quantitative" measurement at the present stage. We believe that improving sensor performance is the next challenge to be solved for quantitative Cl^- ion detection.

Conclusion

In this study, we developed a hydrogel-based sweat Cl^- ion sensing device composed of a fully screen-printed two-electrode system. In particular, the integration of the planar liquid-junction Ag/AgCl reference electrode containing a saturated KCl-based internal electrolyte layer into the device enabled the potentiometric monitoring of sweat Cl^- ion extraction even with a drastic change in the Cl^- ion concentration in the hydrogel. We plan to apply this sensor to other low-concentration sweat components, such as D-glucose (several μM), ammonium ions (several hundred μM), and immune compounds (in the concentration range from pM to nM) for advanced healthcare [30].

However, as discussed above, subtle leakage of Cl^- ions from the electrolyte layer of the planar liquid-junction Ag/AgCl reference electrode into a small volume of hydrogel pad may in some cases affect the sensing reproducibility. Other kinds of silver salts, such as $AgSO_4$ and $AgNO_3$, are also candidates for the reference electrode material to achieve a Cl^- ion free electrochemical cell. Moreover, fine-tuning the PEG content in the PU-based liquid junction will be beneficial in suppressing electrolyte leakage from the reference electrode, while the setup time should also be minimized. Additionally, the quantification of the original sweat analyte concentration is another issue to be solved because we cannot quantify sweat volume extracted in the agarose hydrogel at the present stage. We are considering some strategies to improve quantification, such as the normalization of the detected electrochemical signal using control markers extracted in the same agarose gel, in which concentrations could change depending on the perspiration volume.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00216-021-03156-3.

Funding This work was partly supported by the Center of Innovation Program (COI) from the Japan Science and Technology Agency, JST (JPMJCE1312 and R02W05), and by the Leading Initiative for Excellent Young Researchers (LEADER), a Grant-in-Aid for Scientific Research C (18K04910) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, the YU-COE (C), a research grant from Yamagata University, Japan, and the Foundation for Technology Promotion of Electronic Circuit Board.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the Ethics Committee approved the protocol of the Faculty of Medicine, Yamagata University (Approval No. 30–7).

References

1. Tamhane M, Cabrera-Ghyori S, Abelian G, Viswanath V. Review of biomarkers in ocular matrices: challenges and opportunities. Pharm Res. 2019;36:40.
2. Moreddu R, Vigolo D, Yetisen AK. Contact lens technology: from fundamentals to applications. Adv Healthcare Mater. 2019;8:1900368.
3. Viswanath B, Choi CS, Lee K, Kim S. Recent trends in the development of diagnostic tools for diabetes mellitus using patient saliva. Trends Anal Chem. 2017;89:60–7.
4. Gug IT, Tertis M, Hosu O, Cristea C. Salivary biomarkers detection: analytical and immunological methods overview. Trends Anal Chem. 2019;113:301–16.
5. Tan WS, Tan WP, Tan MY, Khetrapal P, Dong L, de Winter P, et al. Novel urinary biomarkers for the detection of bladder cancer: a systematic review. Cancer Treat Rev. 2018;69:39–52.
6. Bidin MZ, Shah AM, Stanslas J, Seong CLT. Blood and urine biomarkers in chronic kidney disease: an update. Clin Chim Acta. 2019;495:239–50.
7. Baker LB. Physiology of sweat gland function: the roles of sweating and sweat composition in human health. Temperature. 2019;6:211–59.
8. Mena-Bravo A, Luque de Castro MD. Sweat: a sample with limited present applications and promising future in metabolomics. J Pharm Biomed Anal. 2014;90:139–47.
9. Chung M, Fortunato M, Radacsi N. Wearable flexible sweat sensors for healthcare monitoring: a review. J R Soc Interface. 2019;16:20190217.
10. Sonner Z, Wilder E, Heikenfeld J, Kasting G, Beyette F, Swaile D, et al. The microfluidics of the eccrine sweat gland, including biomarker partitioning, transport, and biosensing implications. Biomicrofluidics. 2015;9:031301.
11. Rock J, Makholm L, Eickhoff J. A new method of sweat testing: the CF Quantum® sweat test. J Cyst Fibros. 2014;13:520–7.
12. Brasier N, Eckstein J. Sweat as a source of next-generation digital biomarkers. Digit Biomark. 2019;3:155–65.
13. Dam VAT, Zevenbergen MAG, van Schaijk R. Flexible chloride sensor for sweat analysis. Proc Eng. 2015;120:237–40.

14. Bates GP, Miller VS. Sweat rate and sodium loss during work in the heat. J Occup Med Toxicol. 2008;3:4.
15. McKone EF, Velentgas P, Swenson AJ, Goss CH. Association of sweat chloride concentration at time of diagnosis and CFTR genotype with mortality and cystic fibrosis phenotype. J Cystic Fibrosis. 2015;14:580–6.
16. Legner C, Kalwa U, Patel V, Chesmore A, Pandey S. Sweat sensing in the smart wearables era: towards integrative, multifunctional and body-compliant perspiration analysis. Sens Actuators A: Phys. 2019;296:200–21.
17. Kim J, Campbell AS, de Ávila BE, Wang J. Wearable biosensors for healthcare monitoring. Nat Biotechnol. 2019;37:389–406.
18. Zhang Y, Guo H, Kim SB, Wu Y, Ostojich D, Park SH, et al. Passive sweat collection and colorimetric analysis of biomarkers relevant to kidney disorders using a soft microfluidic system. Lab Chip. 2019;19:1545–55.
19. Bandodkar AJ, Butruf P, Choi J, Lee K, Sekine Y, Reeder JT, et al. Battery-free, skin-interfaced microfluidic/electronic systems for simultaneous electrochemical, colorimetric, and volumetric analysis of sweat. Sci Adv. 2019;5:eaav3294.
20. Choi J, Bandodkar AJ, Reeder JT, Ray TR, Turnquist A, Kim SB, et al. Soft, skin-integrated multifunctional microfluidic systems for accurate colorimetric analysis of sweat biomarkers and temperature. ACS Sens. 2019;4:379–88.
21. Anastasova S, Crewther B, Bembnowicz P, Curto V, Ip HMD, Rosa B, et al. A wearable multi-sensing patch for continuous sweat monitoring. Biosens Bioelectron. 2017;93:139–45.
22. Shay T, Dickey MD, Velev OD. Hydrogel-enabled osmotic pumping for microfluidics: towards wearable human-device interfaces. Lab Chip. 2017;17:710–6.
23. Sekine Y, Kim SB, Zhang Y, Bandodkar AJ, Xu S, Choi J, et al. A fluorometric skin-interfaced microfluidic device and smartphone imaging module for in situ quantitative analysis of sweat chemistry. Lab Chip. 2018;18:2178–86.
24. Hauke A, Simmers P, Ojha YR, Cameron BD, Ballweg R, Zhang T, et al. Complete validation of a continuous and blood-correlated sweat biosensing device with integrated sweat stimulation. Lab Chip. 2018;18:3750–9.
25. Gao W, Emaminejad S, Nyein HYY, Challa S, Chen K, Peck A, et al. Fully integrated wearable sensor arrays for multiplexed in situ perspiration analysis. Nature. 2016;529:509–14.
26. Cazalé A, Sant W, Ginot F, Launay JC, Savourey G, Revol-Cavalier F, et al. Physiological stress monitoring using sodium-ion potentiometric microsensors for sweat analysis. Sens Actuators B: Chemical. 2016;225:1–9.
27. Emaminejad S, Gao W, Wu E, Davies ZA, Nyein HYY, Challa S, et al. Autonomous sweat extraction and analysis applied to cystic fibrosis and glucose monitoring using a fully integrated wearable platform. Proc Natl Acad Soc. 2017;114:4625–30.
28. Kim J, Sempionatto JR, Imani S, Hartel MC, Barfidokht A, Tang G, et al. Simultaneous monitoring of sweat and interstitial fluid using a single wearable biosensor platform. Adv Sci. 2018;5:1800880.
29. Nagamine K, Mano T, Nomura A, Ichimura Y, Izawa R, Furusawa H, et al. Noninvasive sweat-lactate biosensor employing a hydrogel-based touchPad. Sci Rep. 2019;9:10102.
30. Nagamine K, Nomura A, Ichimura Y, Izawa R, Sasaki S, Furusawa H, Matsui H, Tokito S. Printed organic transistor-based biosensors for non-invasive sweat analysis. Anal Sci. 2019, in press.
31. Lin S, Wang B, Zhao Y, Shih R, Cheng X, Yu W, et al. Natural perspiration sampling and in situ electrochemical analysis with hydrogel micro-patches for user-identifiable and wireless chemo/biosensing. ACS Sens. 2020;5:93–102.
32. Fukuda K, Someya T. Recent progress in the development of printed thin-film transistors and circuits with high-resolution printing technology. Adv Mater. 2017;29:1602736.
33. Ihalainen P, Määttänen A, Sandler N. Printing technologies for biomolecule and cell-based applications. Int J Pharm. 2015;494:585–92.
34. Gonzalez-Macia L, Morrin A, Smyth MR, Killard AJ. Advanced printing and deposition methodologies for the fabrication of biosensors and biodevices. Analyst. 2010;135:845–67.
35. Sophocleous M, Atkinson JK. A review of screen-printed silver/silver–chloride (Ag/AgCl) reference electrodes potentially suitable for potentiometric environmental sensors. Sens Actuators A. 2017;267:106–20.
36. Shitanda I, Komoda M, Hoshi Y, Itagaki M. Screen-printed paper-based three-electrode system with a long-term stable and an instantaneously usable reference electrode. Chem Lett. 2018;47:1502–4.
37. Komoda M, Shitanda I, Hoshi Y, Itagaki M. Fabrication and characterization of a fully screen-printed Ag/AgCl reference electrode using silica gel inks exhibiting instantaneous usability and long-term stability. Electrochemistry. 2019;87:65–9.
38. Komoda M, Shitanda I, Hoshi Y, Itagaki M. Instantaneously usable screen-printed silver/silver–sulfate reference electrode with long-term stability. Electrochem Commun. 2019;103:133–7.
39. Sengwa RJ, Dhatarwal P, Choudhary S. Role of preparation methods on the structural and dielectric properties of plasticized polymer blend electrolytes: correlation between ionic conductivity and dielectric parameters. Electrochim Acta. 2014;142:359–70.
40. Zhu W, Wang X, Yang B, Tang X. A novel ionic-conduction mechanism based on polyurethane electrolyte. J Polym Sci B Polym Phys. 2001;39:1246–54.
41. Gonzalo-Ruiz J, Mas R, de Haro C, Cabruja E, Camero R, Alonso-Lomillo MA, et al. Early determination of cystic fibrosis by electrochemical chloride quantification in sweat. Biosens Bioelectron. 2009;24:1788–91.
42. Prompt CA, Quinto PM, Kleeman CR. High concentrations of sweat calcium, magnesium and phosphate in chronic renal failure. Nephron. 1978;20:4–9.
43. Cole DEC, Boucher MJ. Increased sweat sulfate concentrations in chronic renal failure. Nephron. 1986;44:92–5.
44. Weller R, Pattullo S, Smith L, Golden M, Ormerod A, Benjamin N. Nitric oxide is generated on the skin surface by reduction of sweat nitrate. J Invest Dermatol. 1996;107:327–31.