Electronic Supplementary Material (ESI) for Analytical Methods.

Colorimetric Sweat Analysis Using Wearable Hydrogel Patch Sensors for Detection of Chloride and Glucose

Tuqiang Li[a], Qiaoqiao Zhu[a], Xiaofeng Chen[b]*, Ying Fu[c], Caizhi Liao[a]*

a Creative Biosciences (Guangzhou) Co., Ltd, Guangzhou, PR China.

b School of Environmental and Geographical Sciences, Shanghai Normal University, Shanghai, 200234, PR China.

c Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK.

Corresponding author

Prof. Xiaofeng Chen

Shanghai Normal University, Shanghai, China.

Email*: xiaofengchen@shnu.edu.cn* (X.F C)

Prof. Ying Fu

University of Strathclyde, Glasgow, UK.

Email*: ying.fu@strath.ac.uk* (Y F)

Dr. Caizhi Liao

Creative Biosciences, Co. Ltd., Guangzhou, China

Email*: liaocaizhi@creativebio.cn* (C.Z L)

S1. Sweat Sample Collection Ethical Framework

This study recruited 12 healthy subjects, among which 1 subject was withdrawn amid this study due to personal issues. All subjects have given their informed consent for participation in the research study. All subjects have performed Covid-19 tests, with negative results reported. The study protocol and the informed consent form were both approved by the Ethics Committee of the Institute (SZP202e01896). This study is conducted in accordance with the Declaration of Helsinki and Technical Guidance Series for WHO Prequalification – Diagnostic Assessment: Establishing Stability of In Vitro Diagnostic Medical Devices, Guidelines for the Safe Transport of Diagnostic Specimens by National Medical Products Administration (NMPA, 2020 Edition). Access to information about individual participants has been restricted to the researcher, and any staff on a need-to-know basis. Specific care has been taken to avoid breaches of confidentiality in which this information is divulged to anyone else.

S2. Hydrogel Patch Preparation

Agarose gel (3%) based hydrogel patches were prepared for colorimetric sensing of sweat analytes: Buffer was added to a flask that is 2.5 to 4 times the volume of gel solution (at room temperature). The calculated amount of powder then was introduced while stirring vigorously with a Teflon-coated stir bar, so the agarose could be dispersed uniformly. The hydrogel solution was kept stirring for 5 minutes to hydrate the agarose. Then the mixed hydrogel solution was transferred to the flask to a microwave oven and heat it at 100% power (1.5 kW) using 30-second intervals. Noting that during each interval, the powder-solution mixture was gently swirled to resuspend the agarose. The cycle of heating and swirling was continued until the agarose is completely dissolved when no visible particles are visually observed. Cool the solution to 50-60°C before pouring the gel. Finally, slowly pour the solution into the patch mold (diameter: 1 cm, 4 mm deep) without generating bubbles. Chill the gel for 30 minutes before peeling the hydrogel patches from the mode, see **Figure S1**.

S3. Preparation of Microfluidic Sweat Collection Systems

The self-designed microfluidic sweat collection systems were prepared with SYLGARD™ 184 Silicone Elastomer Kit: First, weigh 10g of the large bottle solution (polydimethylsiloxane elastomer solution) and 1g of the small bottle solution (curing agent). Gradually add the curing agent into the polydimethylsiloxane elastomer solution while stirring, followed by a thorough mixing (glass stick) to obtain a mixture with bubbles. Transfer this mixture to a vacuum chamber (~30 mins) to fully remove the bubbles. Then use a pipette gun or a syringe to draw 1 mL of the mixed solution (note: suck slowly to prevent air bubbles) and drop it onto the mold (about 750μL can be fully molded). If air bubbles are generated during this process, continue to vacuum until the air bubbles disappear.

Next, transfer to an oven at 50~80°C to dry (about 2~3h) until the solution becomes solidified (Note: There is no sticky feeling when touching or pressing with tweezers. Then take out the excess part and use a puncher to punch a hole in the center of PDMS, and finally assemble it with the hydrophilic film with designated holes. Noting that the center of the hydrophilic membrane is not perforated, Only the PDMS film has a punched center hole, and the diameter of the center hole is smaller than that of outer holes. For the final three-layer assembly procedure, the grooved surface side and the hydrophilic film side are required to align with holes and the grooves patterns using clean tweezers, see **Figure S3**.

S4. Characteristics of Hydrogel Patch

For practical wearable sweat analysis, the hydrogel patches directly contact the human skin to absorb the sweat and induce colorimetric reactions. Therefore, the microscopic structural features of the hydrogel path play a vital role in realizing real-time analysis. **Figure S2a** shows the hydrogel surface under an optical microscope. The hydrogel patches display an unsmoothed surface (R_a~13 μm) that is similar to that of human skin (R_a~15 μm)(1). This unique surface morphology could improve seamlessness when the hydrogel patch adhered to the skin for measurement(2).

Then SEM was performed to further investigate the morphological properties of the hydrogel patches. As shown in the surface morphology graph (**Figure S2b**), abundant holes, with an opening size from several hundred nm to dozens of μm, were scattered on the surface. These surface opening holes allow the sweat liquid to readily enter the hydrogel patches to induce a corresponding colorimetric reaction. The cross-section view of the hydrogel patch was also presented. **Figure S2c** displays the cross-section view of the -80 °C frozen hydrogel film. The microchannels inside are randomly interconnected and can only be ambiguously viewed as the water molecules are still locked within the swollen hydrogel. These internal microchannels provide two critical roles: I. Transportation pathways for the absorbed sweat; II. Reaction chamber for the colorimetric reactions(3). To get a better insight into these critical internal microchannel structures, we vacuumed and heated the hydrogel patches to fully remove the water molecules to perform the cross-section morphology study. As demonstrated in **Figure S2d**, clear and sharp microchannels are patterned anisotropically within the hydrogels.

Absorbing the sweat quickly is the prerequisite for a feasible real-time sweat analysis system. Therefore, the interfacial hydrogel needs to show superior hydrophilicity. We dropped water onto the surface of a prepared hydrogel patch and performed the contact angle measurement. A near-zero contact angle was recorded, indicating that the prepared hydrogel patch possesses superb hydrophilicity that is determined both by the nature of the gel and the processing scheme(4).

To further evaluate how quickly the sweat can be absorbed and transported within the hydrogel, we carried out the sweat diffusion tests. As the sweat is colorless, we used dyed water to visualize the process. Firstly, a small amount of purple-dyed water (1 μL) was added to the hydrogel patch (diameter: 5 cm, 3 mm deep). Once stabilized, the purple-dyed water was roughly confined to the center region, simulating the addition of colorimetric assay. Then, a larger amount of blue-dyed water (10 μL) was introduced to simulate the sweat-absorbing and sweat-transporting process. As shown in **Figure S2g**, the simulated sweat (blue-dyed water) could easily absorb and rapidly transport within the hydrogel. Within the first 20 s, the simulated sweat has fully mingled with the simulated colorimetric assay reagents and thoroughly penetrates the whole hydrogel patch. Our prepared hydrogel patches hold great potential for real-time sweat analysis.

S5. Colorimetric Sensing Mechanisms

Cl^- Sensing Mechanism

$$\mathrm{Hg-2,4,6-Tris(2-pyridyl)-\ s-triazine\ (TPTZ)} + Fe^{2+} \xrightarrow{Cl^-} HgCl + \mathrm{Fe-TPTZ}$$

The chloride assay provides a simple and direct procedure for measuring chloride in a variety of samples, including blood, urine and sweat. Chloride concentration is determined by a competition reaction between Hg^{2+} and Fe^{2+} for 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ). The preferred Hg-TPTZ complex exhibits no color. In the presence of chloride, Hg^{2+} forms $HgCl_2$, which precipitates, allowing TPTZ to complex with Fe^{2+}. The Fe-TPTZ complex results in a colorimetric product proportional to the chloride present(1, 5).

Glucose Sensing Mechanism:

$$Gluose + ATP \xrightarrow{Hexokinase} Glu\cos e - 6 - Phosphate + ADP$$
$$G6P + NAD \xrightarrow{G6PDH} 6 - Phosphogluconate + NADH$$

Glucose is phosphorylated by adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase. Glucose-6-phosphate (G6P) is then oxidized to 6-phospho-gluconate in the presence of oxidized nicotinamide adenine dinucleotide (NAD), in a reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PDH). During this oxidation, an equimolar amount of NAD is reduced to NADH(6, 7). The consequent colour variations (increase in absorbance at 340 nm) are directly proportional to glucose concentration.

S6. Photo Capturing Setups

To ensure consistency, we have built a light box to capture the images for analysis (See **Figure S4**). To counter the light interference from the lab environment, a strong Microscope LED Polarized Ring Light (MPL108, AOSVI) was assembled on the top of light box. In such setting, the background light would be remained unchanged throughout this work. In photo capturing, the calorimetric hydrogel patch sensors were transferred to the marked sample loading region for analysis. On the other hand, the photo capturing settings of the software/Phone also play a critical role. To improve the reliability of this study, the photo-capturing settings were fixed (see **Table S1**) throughout this work. Based on our experiment results, our prepared hydrogel colorimetric sensors showed optimal performance in these photo-capturing settings. To help readers better understand the process, this figure and photo capturing setting tables were also added to the Supplementary File.

As stated above, the background light remains unchanged throughout this study. Therefore, the impacts caused by background light variations would be negligible. Even though, we still believe a blank control should be coupled with each measurement. When capturing images throughout our work, we all included a companion blank control before capturing the image to further substrate potential background light variations. Results revealed that light variations throughout our work were negligible.

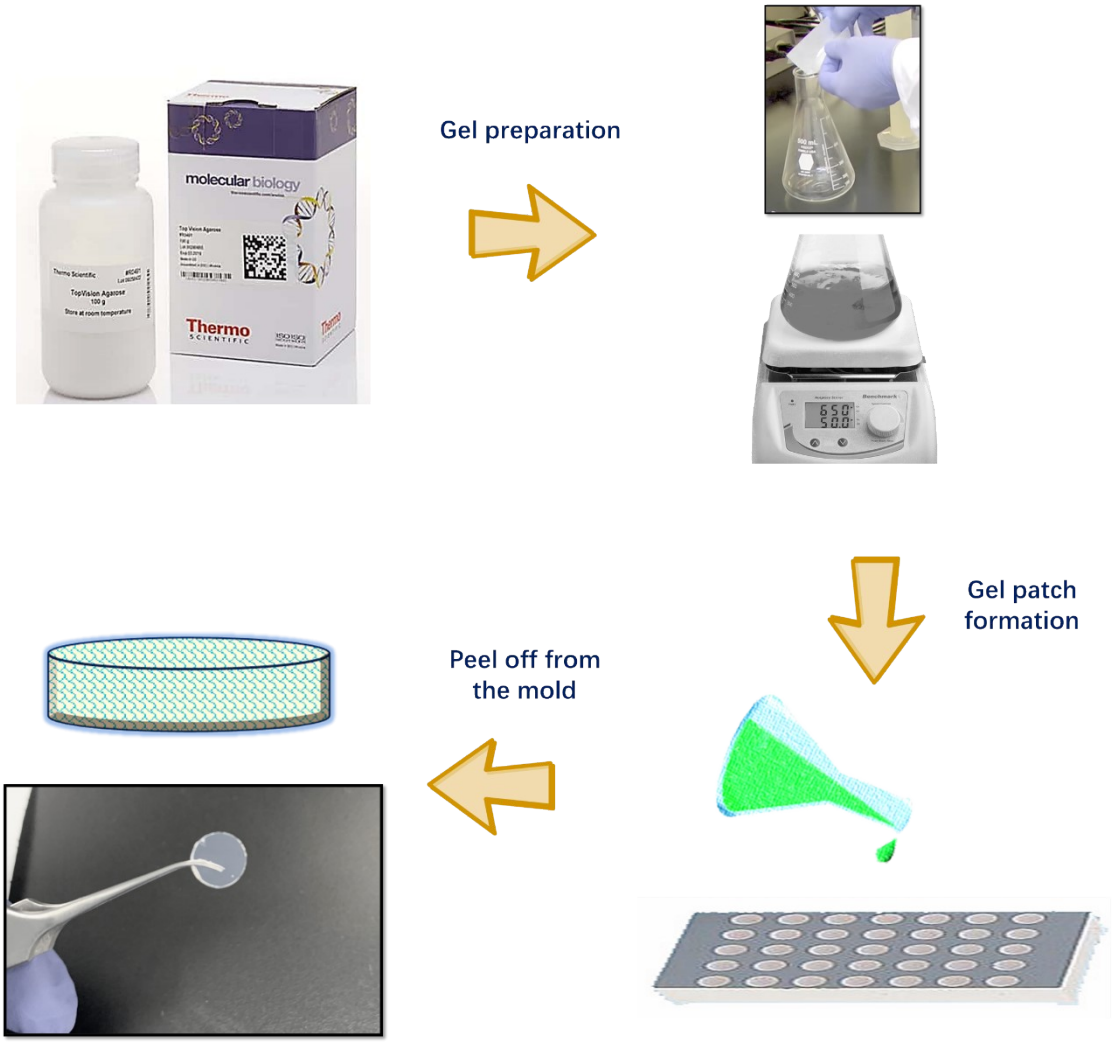


Figure S1. Schematic figures show the preparation of raw hydrogel patches.

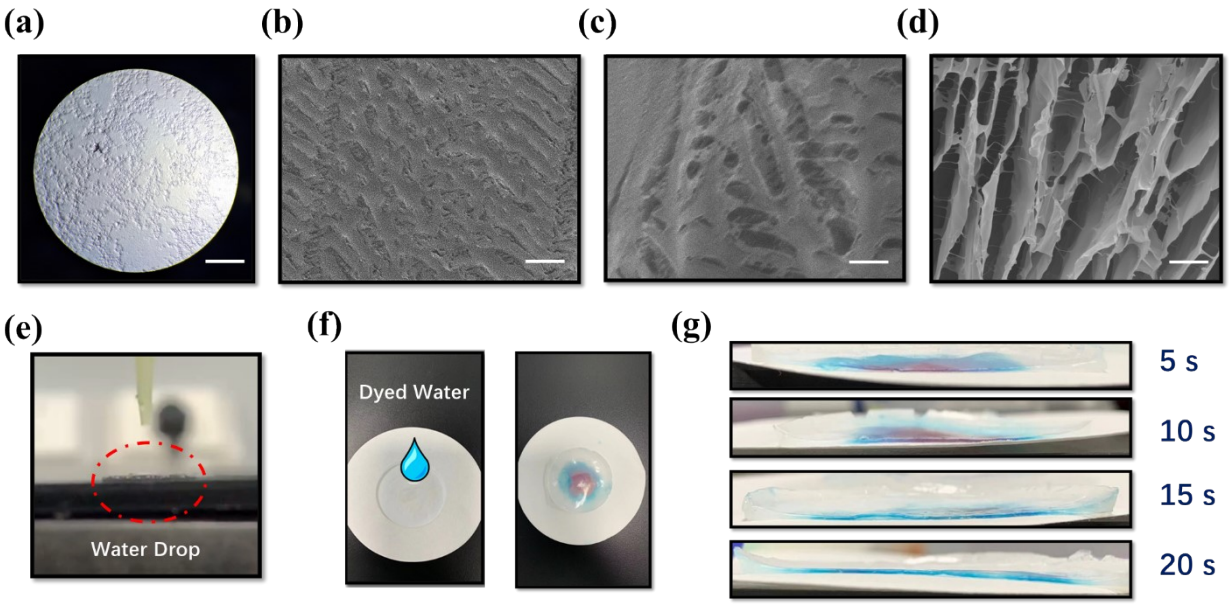


Figure S2. Characterizations of hydrogel patches: (a) Optical image of the surface morphology of the hydrogel patch, and the scale bar is 100 μm. (b) SEM surface morphology image of hydrogel patch (-80 °C frozen), and the scale bar is 100 μm. (c) SEM cross-section morphology image of hydrogel patch (-80 °C frozen), and the scale bar is 10 μm. (d) SEM cross-section morphology image of hydrogel patch (vacuum heated dried), and the scale bar is 10 μm. (e) View of the contact angel after water dropped onto the hydrogel patch. (f) Dyed water dropped to an extended hydrogel patch to visualize the sweat absorption and diffusion process within the hydrogel patch. (g) Cross-section colored view of hydrogel patch after dyed water was introduced, at times intervals of 5 s, 10 s, 15 s, and 20 s.

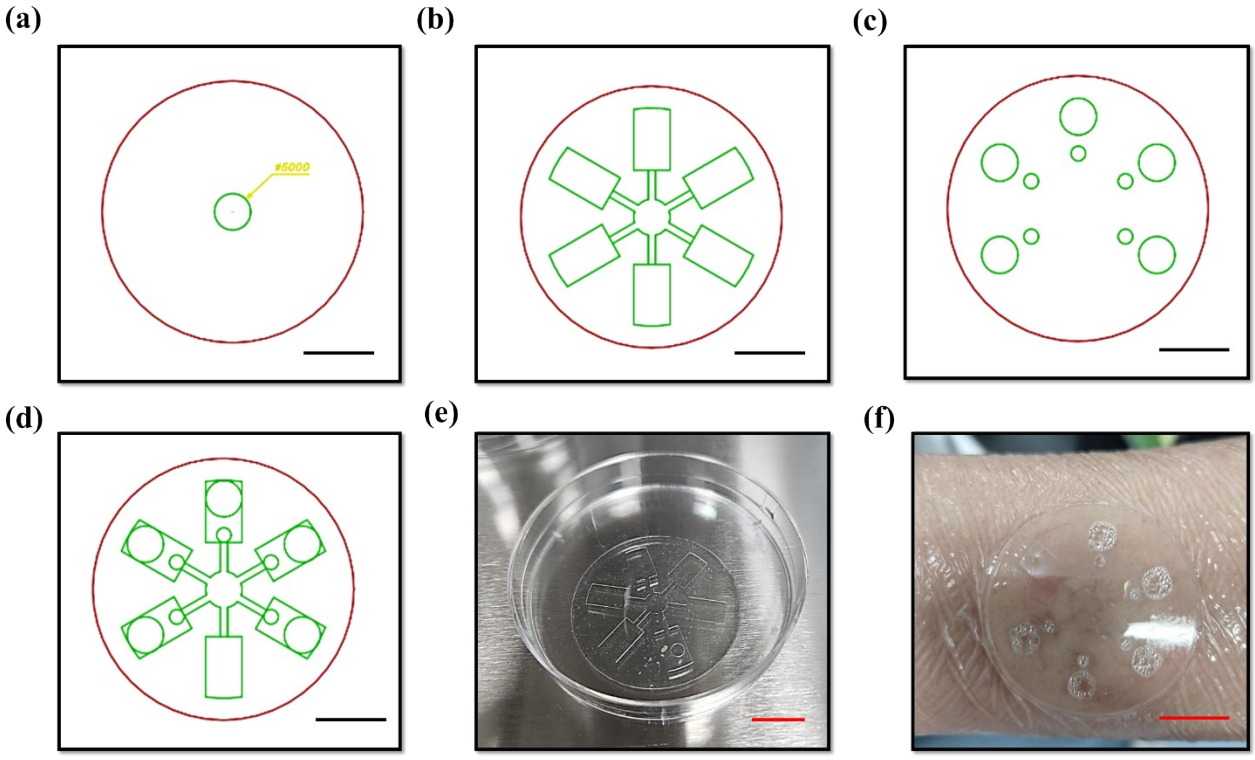


Figure S3. (a) to (c). Schematic diagram (Auto CAD 2021) of three composition PDMS layer parts for assembly. Scale bar: 1 cm. (d). Schematic diagram (Auto CAD 2021) of a three-layer assembled microfluidic sweat collection system. Scale bar: 1 cm. (e). Picture of a three-layer assembled microfluidic sweat collection system. Scale bar: 1 cm. (f) Picture of a microfluidic sweat collection system attached to sweaty skin to collect sweat samples. The sweat is concentrated in the sweat storage chambers. Scale bar: 1 cm.

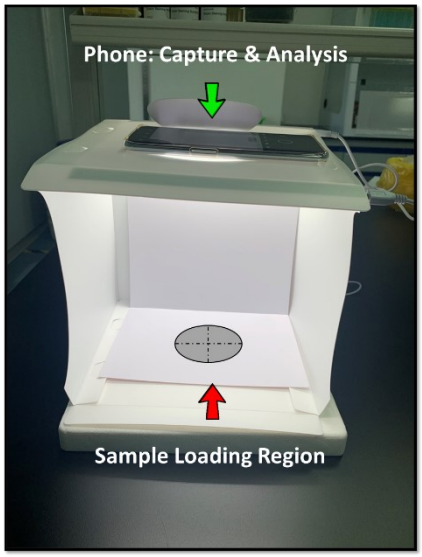
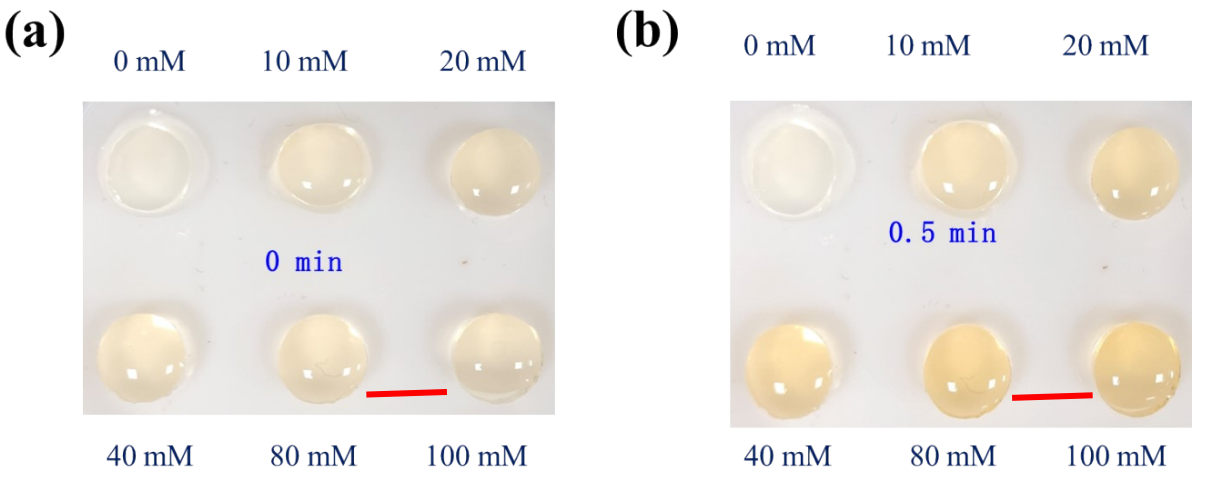
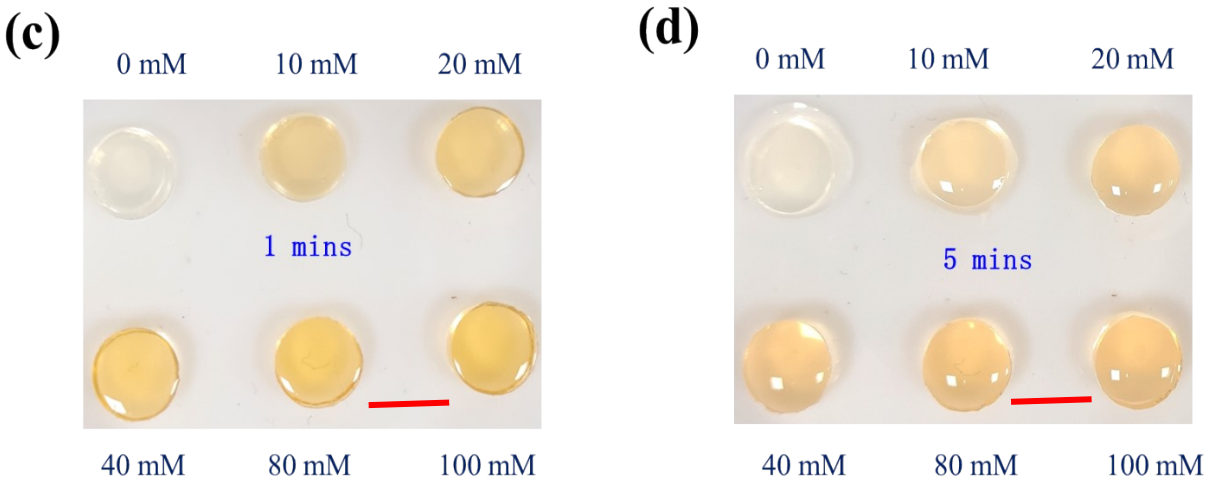


Figure S4. Self-built light box equipped with ring LED light source for image capturing.

Table S1. Fixed photo capturing parameters

Capturing Parameters	Value
Exposure time	6 ms
Exposure compensation	56
Exposure gain	0
Background Red	119
Background Green	102
Background Blue	73
Sharpen	10
Noise reduction	8
Saturation	50
Gamma	10
Contrast	50
Brightness	50


(a)
0 mM
10 mM
20 mM
0 min
40 mM
80 mM
100 mM
(b)
0 mM
10 mM
20 mM
0.5 min
40 mM
80 mM
100 mM


(c)
0 mM
10 mM
20 mM
1 mins
40 mM
80 mM
100 mM
(d)
0 mM
10 mM
20 mM
5 mins
40 mM
80 mM
100 mM

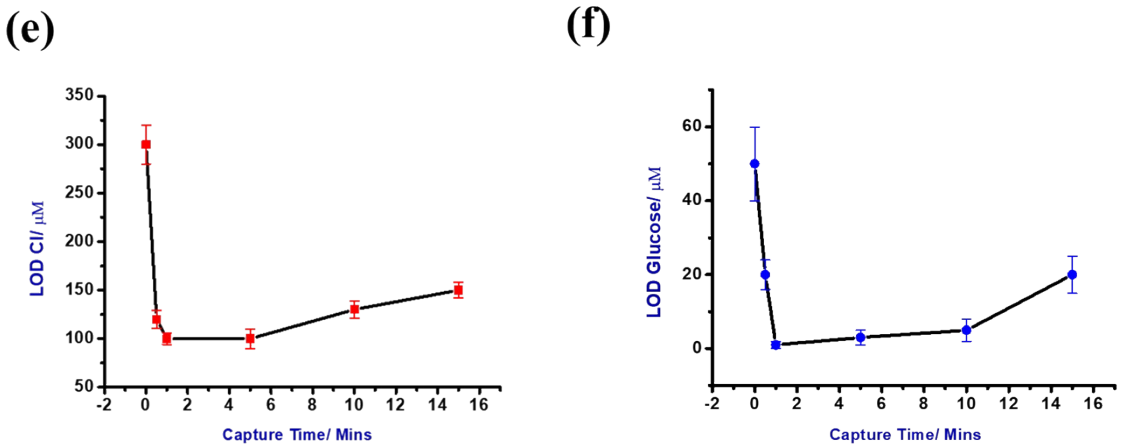

(e)
LOD Cl/ μM
350
300
250
200
150
100
50
-2
0
2
4
6
8
10
12
14
16
Capture Time/ Mins
(f)
LOD Glucose/ μM
60
40
20
0
-2
0
2
4
6
8
10
12
14
16
Capture Time/ Mins

Figure S5. Color variations of the Cl^--responsive hydrogel patch added with an increased amount of Cl^- (0 mM, 10 mM, 20 mM, 40 mM, 80 mM, 100 mM) photoed at 0 min (a), 0.5 min (b), 1 min (c), and 5 mins (d). Scale bar: 0.5 cm. (e) The limit of detection of Cl- sensor vs the image capture time. (f) The limit of detection of glucose sensor vs the image capture time

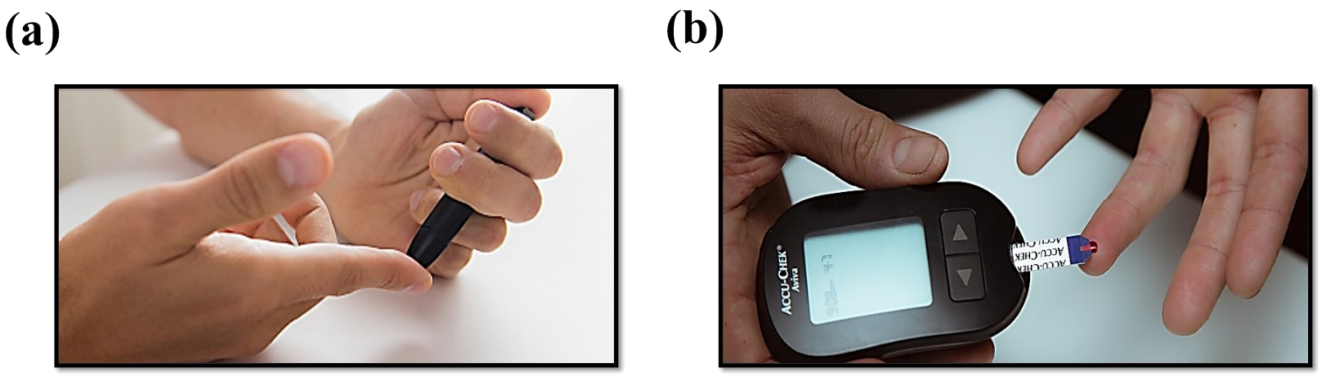


Figure S6. Schematic pictures of the measurement of blood glucose levels in this work. (a) Use a sharp need to prick the finger to generate blood sample. (b) Transfer the blood sample to the glucose test trips for blood glucose analysis.

Table S2. Manufacturing cost summary for each type of colorimetric hydrogel sensor

Note: Manufacturing cost per sensor was calculated based on the production of 1000 hydrogel-based sensors assembled on the medical dressing. The cost of all involved production machines was included. For cost calculation, machines are assumed to depreciate by 50% in the 1st year, and 20% per year in subsequent years.

Chlorine-responsive Sensor	Preparation of Agarose hydrogel patch (Raw)	~ 0.02
	Addition of Chlorine colorimetric assay kit reagents	~ 0.05
	Assembly on Medical Dressing substrate	~ 0.2
	Miscellaneous	~ 0.5
	Overall Cost (Chlorine)	**~ 0.77**
Glucose-responsive Sensor	Preparation of Agarose hydrogel patch (Raw)	~ 0.02
	Addition of Chlorine colorimetric assay kit reagents	~ 0.09
	Assembly on Medical Dressing substrate	~ 0.2
	Miscellaneous	~ 0.4
	Overall Cost (Glucose)	**~ 0.71**

Reference

1. Gao W, Ota H, Kiriya D, Takei K, Javey A. Flexible Electronics toward Wearable Sensing. Acc Chem Res. 2019;52(3):523-33.
2. Liao C, Wuethrich A, Trau M. A material odyssey for 3D nano/microstructures: two photon polymerization based nanolithography in bioapplications. Appl Mater Today 2020;19:100635.
3. Liang Y, He J, Guo B. Functional Hydrogels as Wound Dressing to Enhance Wound Healing. ACS Nano. 2021;15(8):12687-722.
4. Li L, Lin Q, Tang M, Duncan AJE, Ke C. Advanced Polymer Designs for Direct-Ink-Write 3D Printing. Chemistry. 2019;25(46):10768-81.
5. Emaminejad S, Gao W, Wu E, Davies ZA, Yin Yin Nyein H, 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 Sci U S A. 2017;114(18):4625-30.
6. Vaquer A, Baron E, de la Rica R. Detection of low glucose levels in sweat with colorimetric wearable biosensors. Analyst. 2021;146(10):3273-9.
7. Yue X, Xu F, Zhang L, Ren G, Sheng H, Wang J, et al. Simple, Skin-Attachable, and Multifunctional Colorimetric Sweat Sensor. ACS Sens. 2022;7(8):2198-208.