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Wireless thermometry for real-time temperature recording on thousand-cell level

Can Li, Jianfei Sun*, Qiwei Wang, Weiguo Zhang, and Ning Gu*

***Abstract*— *Objective:* A wireless, multi-channel system for real-time measurement of the cellular-temperature response to drugs is developed. *Methods:* The acquisition system applies a high-precision reference resistor and a low real-time measurement current (below or equal to 0.14 mA) to reduce self-heating via the intermittent measurement. Cells of a small volume cell medium are cultured on the surface of the platinum thermal resistor and subsequently measured in the incubator. *Results:* The resistance resolution of the circuit exhibits 20 mΩ, which corresponds to no more than 0.01 °C. The resistance deviations of each channel are corrected with software compensation. The linearity between the temperature and resistance of the sensors lies above 0.999 in the applied temperature range (30 °C–42 °C). Observations with the scanning electron microscope (SEM) show that the cells grow well on the sensor surface. The latter is composed of a glass glaze, which is nontoxic for organisms. The cell population temperature measurements under norepinephrine action present an obvious temperature rise, which can be the result of the drug binding to the receptors on cell membrane thus promoting a cationic inflow. *Conclusion:* The platinum sensor and multi-channel acquisition system can be used to determine the temperature changes of cells in their original state. *Significance:* The wireless, real-time, high-throughput temperature detection method is particularly suitable to evaluate the thermogenic ability of growing cells that interact with other matter or organisms. The proposed method can help to explore thermal changes in cell populations, intercellular connections, and social connections of cells.**

***Index Terms*—cell, temperature measurements, real-time, in situ, thermometry**

I. INTRODUCTION

The temperature is a key parameter for life, and cells are the fundamental unit of life. All processes in the evolution of life are accompanied by energy changes, which are expressed as heat/temperature changes. It is vitally necessary to measure the cellular temperature to fully understand life sciences [1]. Mitochondria (and chloroplast in plants) are the power house of the cell and store and release energy in the form of adenosine triphosphate (ATP) molecules [2]. Heat is generated because many reactions in the energy metabolism, e.g., reactions catalyzed by the mitochondrial respiratory chain and reactions that consume ATP (e.g., Na^+/K^+ ATPase, Ca^{2+} ATPase, and actin–myosin ATPase), are exothermic in the forward direction [3]. Therefore, a variety of methods for the evaluation of cellular or intracellular temperature or thermogenesis have been developed. Luminescence-based thermometry and composite-structure thermometry are the most commonly used cellular-temperature detection methods. For example, Arai et al. monitored temperature changes in mitochondria with Mito thermo yellow as mitochondria-specific dye [4]. Zohar et al. integrated europium (III) thenoyltrifluoro-acetonate, which is a rare-earth chelate and has a good hydrophobic nature helping CHO cell membrane penetration for imaging intracellular heat waves after the activation of the muscarinic receptor m1 by acetylcholine [5]. In addition, many invasive thermometry methods have been developed in recent years, e.g., the thermocouple [6] and resonant thermal sensor [7]. However, several studies have demonstrated that cell culture methodology can significantly affect the cell state. For example, the decrease of the culture temperature results in a rapid decrease in cell proliferation along with the accumulation of cells in the G1 phase [8], and a decrease of the total metabolic rate of the cells as well as live cell count [9]. The cell productivity in a CHO perfusion culture reactor was maximized when pCO2 was maintained in the range of 30–76 mm Hg [10].

To address the problems mentioned above, we developed a real-time method for in situ measurements of the cell population temperature. The method allows to measure the cell group temperature for a normal growing state without doing damage to the cells. Further, the intercellular communication is undisturbed, which is especially important as cells form a complex cellular society [11]. The wireless, real-time, high-throughput temperature detection method allows to monitor the process of cellular interactions with the extracellular matrix in a fast time resolution.

Vascular endothelial cells play a key role in triggering and spreading an arterial inflammatory response. An increased endothelial permeability is the hallmark of inflammatory vascular edema. Inflammatory mediators that bind to heptahelical G protein-coupled receptors trigger an increased endothelial permeability by increasing the intracellular Ca^{2+} concentration ($[Ca^{2+}]$ i) [12]. Increasing the temperature from 22°C to 40°C in mammalian cells transfected with hTRPV3

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Can Li, Qiwei Wang, and Weiguo Zhang are with the School of Biological Sciences & Medical Engineering, Southeast University,

State Key Laboratory of Bioelectronics and Jiangsu Key Laboratory of Biomaterials and Devices, Southeast University, Nanjing, China, and Collaborative Innovation Center of Suzhou Nano Science and Technology, Suzhou 215123, China.

Jianfei Sun is with the School of Biological Sciences & Medical Engineering, Southeast University, Nanjing, China (correspondence e-mail: sunzaghi@seu.edu.cn).

Ning Gu is with the School of Biological Sciences & Medical Engineering, Southeast University, Nanjing, China (correspondence e-mail: guning@seu.edu.cn).

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elevates the intracellular calcium by activating a nonselective cationic conductance [13][14], whereas the amount of heat released during the hydrolysis of ATP can vary depending on whether or not a transmembrane Ca^{2+} gradient is formed across the sarcoplasmic reticulum membrane [15]. Researchers showed that norepinephrine in a certain concentration range contributes to the maintenance of the endothelial barrier in vivo [16][17][18]. Therefore, the norepinephrine action on the endothelial cells can cause an important temperature change, which is meaningful for studying endothelial cells as well as for the treatment of inflammations.

Thus, we choose human microvascular endothelial cells (HMEC-1) for the cell population temperature measurements under norepinephrine action. The applied thin-film platinum resistors exhibit small volumes, a quick response time, high precision (±0.01 °C), high linearity between temperature and resistance in a small temperature range, good long-term stability, and a proper application temperature range (0 °C–150 °C). The sensors are composed of a submicron- or micron-thick platinum film. The attached ceramic substrate with a glass glaze coated on the surface is nontoxic for organisms. Owing to the intermittent measurements (ratio of measurement time and rest time is 1:1) and the low current flowing through a platinum resistor (below 0.14 mA), self-heating by the thermal resistance can be reduced and a precise measurement circuit is obtained. By employing a high-precision resistance acquisition system with a high-precision reference resistor (10 kΩ, 0.005 %, 0.1 ppm) and pulsing in a four-line test mode, a high measurement accuracy for the resistance can be obtained. Each channel is tested by connecting a high-precision reference resistor (1 kΩ, 0.01 %, 5 ppm) to the measurement system, and corrected via software compensation. By adopting wireless technology, the resistance changes of the thermal resistor caused by the temperature changes of the cells in the incubator are transmitted to the computer outside the incubator to be recorded and processed. The thin-film platinum resistor temperature sensors can thus be used to measure small changes in the cell population temperature in the incubator.

The resistance resolution of the circuit is 20 mΩ, which corresponds to 0.01 °C. The linearity between temperature and resistance of the sensors is governed by a value of 0.999 according to the statistical data. Observations with a scanning electron microscope (SEM) show that the cells grow well on the sensor surface. The cell population temperature measurements under norepinephrine action exhibit an obvious temperature rise accompanied by some burrs, which can be caused by the cell activity. The platinum sensor and multi-channel acquisition system can be used to measure cellular-temperature changes under norepinephrine action of cells in their original state. This method is particularly suitable for the evaluation of the thermogenesis of cells under normal conditions in contact with other matter or organisms, e.g., materials, drugs, bacteria, and viruses.

II. EXPERIMENTAL METHOD

We designed and tested the wireless multi-channel system, seeded human microvascular endothelial cells (HMEC-1) on the thin-film platinum resistance PT1000 sensor, stimulated them with norepinephrine, and evaluated the temperature response of the cell populations and cultivation medium simultaneously to remove the norepinephrine effect on the culture medium. Of course, we confirmed the growth of cells on the platinum surface before the temperature measurement with an SEM. The schematic diagram of the experiment is showed in Fig.1a.

A. Circuit design

The platinum resistance temperature sensor exhibits resistance changes with changing temperature[19] and enables high-precision temperature measurements. However, the sensor has the following shortcomings: measurement errors caused by the lead resistance, obvious self-heating caused by high currents, and component drift caused by the environment. Reducing the influence of the lead resistance is of key importance for high-precision measurements [20]. Regarding self-heating, the component-heating can be described by

$$P = I^2 R \quad ,$$

where P, I, and R denote the heat produced per unit time, current flowing through the sensor, and resistance of the sensor, respectively. For the correct measurement of the cell temperature, it is necessary to realize a small enough current that flows through the component. The conventional measurement method for platinum resistor temperature sensors is based on a constant current source (or constant voltage source). Then, the voltage (or current) that varies with the resistance R is determined with

$$R = \frac{V}{I} \quad ,$$

where V and I denote the voltage and current of the sensor, respectively. Therefore, also R can be obtained. The temperature is determined by combining the relation between temperature and resistance of the sensor. In addition, the current flowing through it must be small enough to reduce the self-heating effect. Nevertheless, very small currents are not steady enough for long-time measurements because multiple factors such as noise of the environment and component errors influence the setup. To achieve accurate measurements without obvious self-heating, we designed a circuit that does not depend on the small current flowing through the sensor, but on the ratio of the voltage of the precise reference resistor and voltage of the sensor to be measured. As the high-precision reference resistor is known, the resistance value of the sensor at each moment can be easily obtained (see Fig.1b). According to Fig.1b,

$$\frac{V_1}{V_2} = \frac{R_t}{R_S} \quad .$$

As R_S (10 kΩ, 0.005 %, 0.1 ppm) is known, V_1 and V_2 can be acquired from the circuit to determine R_t. Further, intermittent measurements help to reduce self-heating.

Each resistance value was averaged according to two

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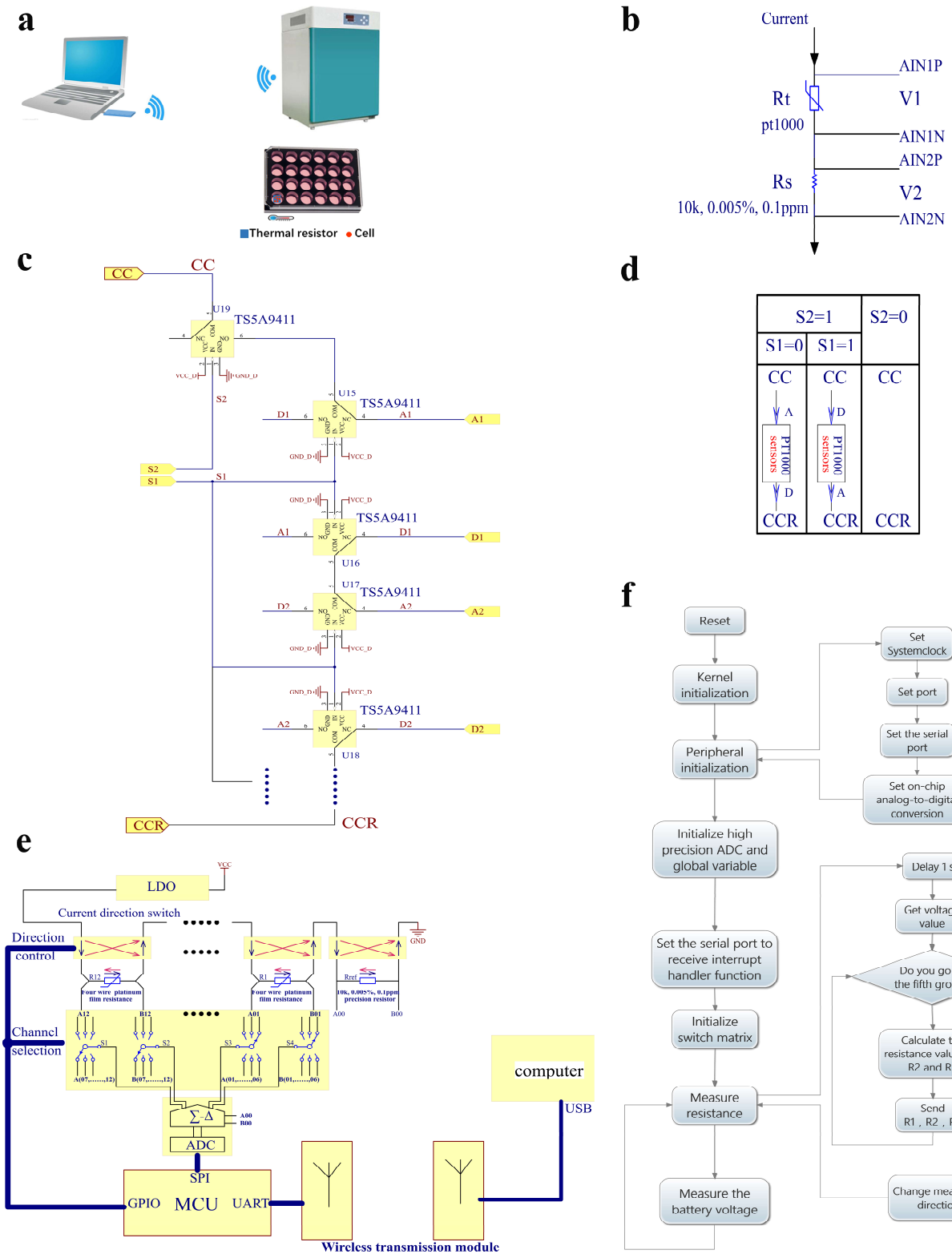

Fig.1. Schematic diagram of experimental setup and system design. a: schematic diagram of experiment. b: schematic diagram of high-precision resistance measurement. c: schematic diagram of current direction-switching circuit. d: parameter description of current direction-switching circuit. e: system hardware design. f: system software design.

measurements with reversed current directions. Thus, measurement errors caused by the environment can be reduced (see Fig.1c and Fig.1d).

The voltage values were obtained using a high-precision and low-noise instrumentation amplifier AD624 (ANALOG DEVICES). At each measurement point, the voltages of a

subgroup containing two channels were obtained sequentially through the switching circuit. Before the measurements, the circuit was opened, then delayed for four seconds. Subsequently, each subgroup was measured. In total, the last process takes nine seconds. Next, the measurement channel was closed to let the circuit sleep for five seconds. The complete above-mentioned measurement of the 12 channels takes 18 s. The data were stored in an MCU and sent out through a wireless transmitter. The wireless receiver connected to the computer receives the data from the wireless transmitter. After conversion processing, the data were stored and then displayed by the software. Fig.1e and Fig.1f present the diagram of the system design and software flow chart. For more detailed circuit information, please refer to the supporting information (Fig.S1 and Fig.S2).

B. Sensor calibration

Before the cell experiment, the PT1000 thin-film thermal resistors (Heraeus; M222; length × width × height: 2.3 mm×2.1 mm×0.9 mm; line length: 10 mm; degree: AA; temperature coefficient: TCR = 3850 ppm/K; accuracy: ±0.01 °C; self-heating coefficient： 0.4 K/mW (at 0 °C); response time in water: 0.4 m/s: $t_{0.5}$ = 0.05 s, $t_{0.9}$ = 0.15 s; response time in air: 2 m/s: $t_{0.5}$ = 3.0 s, $t_{0.9}$ = 10.0 s; measurement range: 0 °C –150 °C; measurement current: 0.1 mA–0.3 mA) were calibrated in a standard water tank (Huzhou Weili instrument factory; HTS-0A; temperature fluctuation ≤ 0.02 °C/10 min; temperature uniformity ≤ 0.02 °C; factory number: 2940) with a secondary standard platinum resistance thermometer with a temperature accuracy of 0.02 °C. We fitted the temperature value (t) and corresponding resistance value (R) in accordance with

$$R = k \times t + b \quad ,$$

where k is the linear coefficient and b the intercept.

C. Device processing

After the PT1000 thin-film thermal resistors were calibrated, they were integrated into a six-well plate. Each well contained two thin-film thermal resistors; one for control and another for tests. Next, the six-well plate with the PT1000 thin-film thermal resistors was sterilized with ethylene oxide and sealed for use.

D. Cell culture and cell observation

Microvascular endothelial cells from the human skin (HMEC-1) were obtained from the American type culture collection (ATCC). The HMEC-1 cells were incubated in a Dulbecco's modified Eagle medium. All media were supplemented with 10 % (vol/vol) fetal bovine serum and 1 % penicillin/streptomycin. All cells were cultured in humidified incubators maintained at 37 °C with 5 % CO_2. After the incubation, the HMEC-1 cells were digested and seeded on the test thin-film thermal resistor surface (10000 cells for each testing sensor). We dripped 20 μl cell medium on the sensor surface. Owing to the liquid surface tension, all cells were seeded onto the sensor surface. After approximately 2 h, when the cells were attached to the sensor surface, a culture medium of suitable volume for cell growth was added. After more than 12 h, the growth status of the cells was investigated. As the sensors are not translucent, the cells could not be observed using light microscopes. Hence, the SEM was selected. Before the observation, the cells were ethanol gradient dehydration and immobilization, as well as dried before observation.

E. Cellular activity under norepinephrine

Before the drug stimulation experiment, the toxicity of the drug to the cells was evaluated. The cells were seeded on a 96-well plate with 5000 cells per well. After 24 h, norepinephrine volumes (Solarbio, SN8550) of different concentrations (0 μM, 0.01 μM, 0.1 μM, 1 μM, 10 μM, 100 μM), diluted into the complete culture medium, were added to the well plate by replacement of culture medium. The cell viability was measured using the CCK-8 method (KeyGEN Biotech, KGA317) after 24h action. The experiment was conducted at least three times.

F. Cell temperature measurement under norepinephrine of 2.5 μM

After the cells were seeded on the sensor surface, the temperature experiments were carried out after 12 h. Before the temperature measurement, the drug was preheated until it reaches the room temperature (25°C). The norepinephrine was added to the cell medium with a final drug concentration of 2.5 μM when the cell reached a steady state (i.e., small temperature fluctuations). The temperatures of the cell suspension ($t_{control}$) and cell ($t_{cell\ with\ suspension}$) were tested simultaneously. The temperature changes of pure cells were calculated in accordance with

$$\Delta t = t_{cell\ with\ suspension} - t_{control} \quad .$$

The experiment is conducted at least three times.

*G. Cell growth and morphological changes under norepinephrin*e

The morphological changes of cells under a norepinephrine concentration of 2.5 μM were observed using a confocal laser microscope system (Olympus Japan; FV1000-D) equipped with a 40× objective (Olympus; UPlanSApo 40/1.35). During image shooting, the norepinephrine was added to the cell medium after 10 min from the start. The optical microscopic images of the cells were then acquired every 10 min. The experiment was conducted at least three times.

H. Cell temperature changes *caused by norepinephrin*e with *different concentrations*

The cells were seeded on the surface of the testing sensors. The temperature experiments were carried out after 12 h. Before the temperature evaluation, the drug was preheated until it reached the room temperature (25°C). At the same time, the sensors were connected to the homemade system and the software was used for recording data. When the temperature reached an equilibrium state (fluctuations below 0.1 °C), norepinephrine was added to the cell medium with concentrations of 0 μM, 0.01 μM, 0.1 μM, 1 μM, 10 μM, and 100 μM. The temperature of the cell suspension ($t_{control}$) and cell ($t_{cell\ with\ suspension}$) were determined simultaneously. The temperature changes Δt of the pure cells were calculated using

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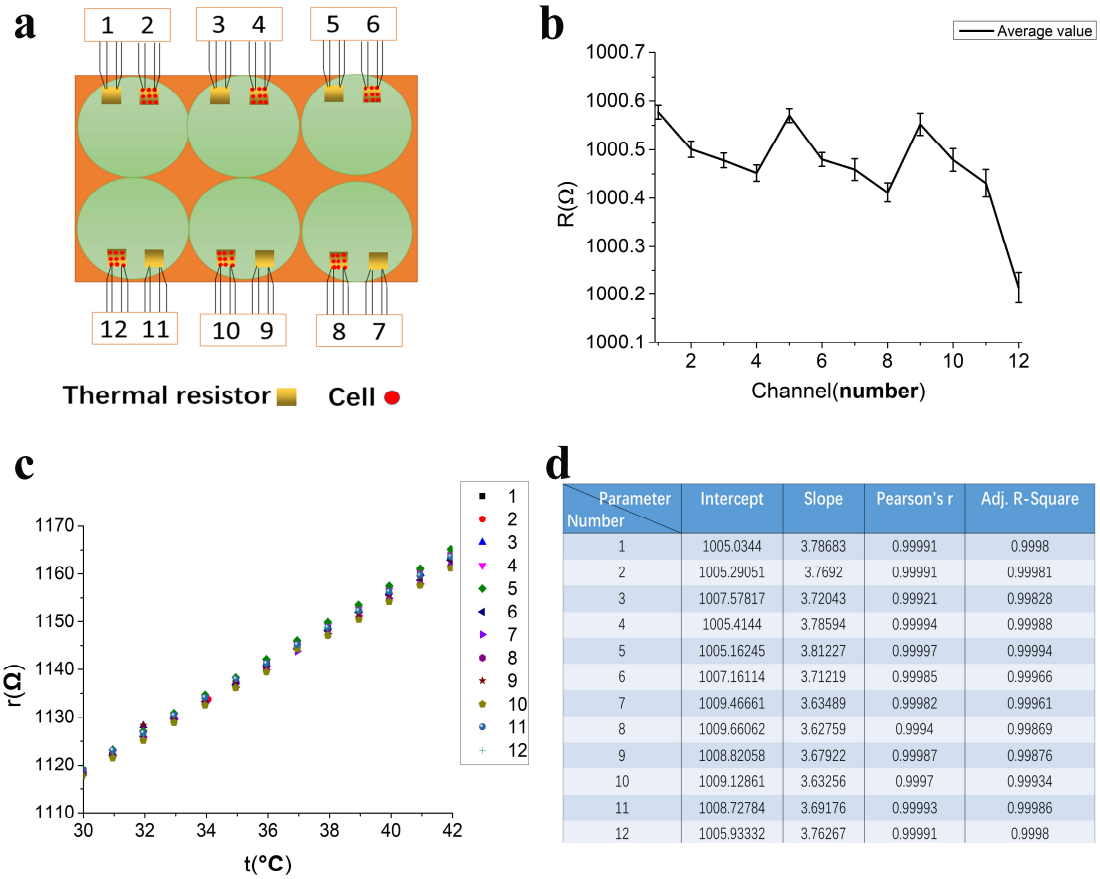

Parameter / Number	Intercept	Slope	Pearson's r	Adj. R-Square
1	1005.0344	3.78683	0.99991	0.9998
2	1005.29051	3.7692	0.99991	0.99981
3	1007.57817	3.72043	0.99921	0.99828
4	1005.4144	3.78594	0.99994	0.99988
5	1005.16245	3.81227	0.99997	0.99994
6	1007.16114	3.71219	0.99985	0.99966
7	1009.46661	3.63489	0.99982	0.99961
8	1009.66062	3.62759	0.9994	0.99869
9	1008.82058	3.67922	0.99987	0.99876
10	1009.12861	3.63256	0.9997	0.99934
11	1008.72784	3.69176	0.99993	0.99986
12	1005.93332	3.76267	0.99991	0.9998

Fig.2. Schematic diagram of the thermal resistor temperature sensor connection and the calibration and test curves of the sensors. a: diagram of thermal resistor integrated into pole plate for temperature measurements. b: average deviation of each channel relative to high-precision standard resistance (1 k Ω, 0.01 %, 5 ppm). c: resistance versus temperature of platinum resistance sensors. d: linear fitting parameters of sensors in Fig.2c.

$$\Delta t = t_{\text{cell with suspension}} - t_{\text{control}}.$$

The experiment was conducted at least three times.

III. RESULTS AND DISCUSSIONS

A. Device test and sensor calibration

The 12-channel wireless temperature measurement system was produced and tested. The current flowing through the sensor measured between 0.131 mA and 0.134 mA. To reduce errors caused by self-heating, discontinuous measurements were carried out (the duty cycle of each measurement cycle was 50 %). The measurement time for each test for 12 channels was 18 s.

To calculate the resistance deviation of each circuit channel relative to the standard value, each channel was examined for 48 h by connecting a high-precision resistor (1 kΩ, 0.01 %, 5 ppm). The results are shown in Fig.2b (the test was conducted at least three times). The deviations were corrected via software compensation, and the resistance resolution of the circuit was 20 mΩ. The fitting curves of temperature and corresponding resistance in accordance with

$$R = k \times t + b,$$

which are shown in Fig.2c (only part of the R (resistance, Ω)–t (temperature, °C) curves as each sensor was independently calibrated). The values of R and t exhibited a good linearity.

B. Cell culture and cell observation

The SEM observation results are shown in Fig.3a. Fig.3a-1 presents the surface morphology of the sensor without cells, whereas Fig.3a-2 shows the case with cells. According to Fig.3a-2 and Fig.3a-3, which is the local magnification of Fig.3a-2, the cells grew well on the sensor surface. Besides, many links were observed to exist among the cells.

C. Cell activity under norepinephrine

According to the results in Fig.S3, different norepinephrine concentrations (0 μM, 0.01 μM, 0.1 μM, 1 μM, 10 μM) had no obvious effect on the cellular viability after 24 h.

D. Cell population temperature measurement

The results are shown in Fig.3c–Fig.3e. Fig.3c presents the temperature curves of the culture medium (black) and cells (red); Fig.3d shows the temperature changes of the cells relative to those of the culture medium; Fig.3e is the local magnification of the elliptic curve in Fig.3d, which shows the time point of the equilibrium temperature of the cells under added norepinephrine.

According to the results, the temperature of cells elevated by 0.3 °C under the action of norepinephrine. The temperature reached to its maximum after approximately 6 h, and then dropped slightly and remained relatively steady.

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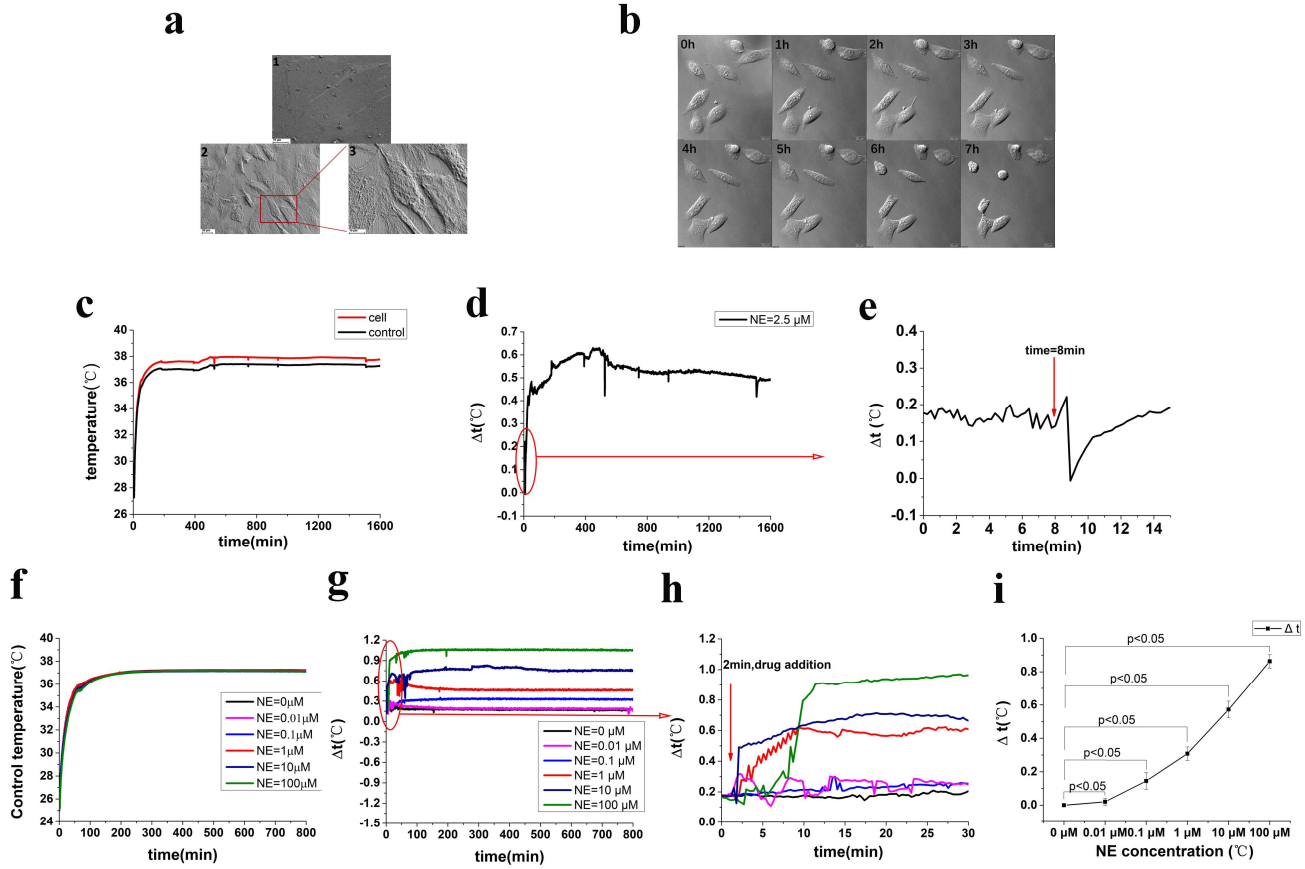

Fig.3. Cell characterization and temperature measurement. a: SEM characterization of sensor surface without and with cells. 1: Surface morphology of sensor without cells; 2: Cells grow well on the sensor surface; 3: Local amplification of Fig.3a-2. b: Morphological changes of cells at different times under norepinephrine action. c: Temperature curves of culture medium and cells under norepinephrine action; the final concentration of norepinephrine is 2.5 μM. d: Temperature changes of cells relative to temperature of culture medium. e: Local amplification of elliptic curve in Fig.3d, which presents the time point and equilibrium temperature of the cells with added norepinephrine. f: Medium temperature for each drug concentration action on cells. g: Temperature changes of cell relative to medium temperature regarding each drug concentration. h: Local amplification of Fig.3g, which presents the detailed temperature changes before and after drug addition. i: Temperature rise compared to drugless condition; Δt is the temperature change of each drug concentration action on the cells minus that of the drugless condition.

E. Cell growth and morphological changes under norepinephrine

Fig.3b shows the hourly images of the cells of 0 h–7 h. During norepinephrine stimulation, the initially regular elliptic cells (Fig.3b, inset for 0 h) lost their distinct shape and shriveled gradually (Fig.3b, inset 1 h–7 h). The decreasing volume of the cell can be caused by the presence of a transmembrane Ca^{2+} concentration gradient. Agents that modify the amount of ATP synthesized from ADP and Pi also modify the amount of heat produced by the hydrolysis of each ATP molecule. For example, in the presence of heparin, less ATP was synthesized, thus more heat was produced [14].

F. Cell temperature changes caused by different norepinephrine concentrations

The results are presented in Fig.3f–Fig.3h. Fig.3f shows the medium temperature of each drug concentration acting on the cells; Fig.3g presents the temperature changes of cells relative to the medium temperature changes, whereas Fig.3h is a local magnification of Fig.3g, which shows the detailed temperature changes before and after drug addition. According to the curves, different norepinephrine concentrations caused different temperature rises. In general, the cellular temperature increased with increasing drug concentration. The temperature rise relative to the drugless condition was calculated and is presented in Fig.3i. The temperature rises of each concentration (except the condition without norepinephrine) relative to the drugless condition (the concentration of norepinephrine was 0 μM) exhibit significant differences ($P < 0.05$).

Regarding the temperature changes in norepinephrine - stimulated cells, the drug possibly interacts with the α receptor on the cell membrane, which leads to an increasing intracellular Ca^{2+} concentration, thus increasing the cellular temperature. Studies have shown that the binding of α-adrenergic receptors to the hormone can cause an increase of the Ca^{2+} concentration [20]—unlike β-adrenergic receptors that cause the cAMP concentration to rise [21]. It may be due to the opening of the Ca^{2+} channel on the membrane and the entry of extracellular Ca^{2+} into the cell [22] [23]. For example, astroglial cells respond to α-adrenergic receptor stimulations with an increased intracellular calcium amount, which can be mediated by α1- and/or α2-adrenergic receptors. Further, subpopulations of

cerebral-type 1 astroglia exist with respect to α-adrenergic receptor expression [24].

Studies have shown that the temperature rise may open certain cation channels in cells, thereby promoting an ion exchange inside and outside the cells [25][26]. In addition, a Ca^{2+} shock can be used to boost the heat production [27]. For example, by elevating the intracellular concentration of Ca^{2+}, the temperature inside the cell rises[28], which can be caused by the promotion of the ion pump activity and acceleration of respiration rates [29] [30]. Moreover, the elevated cell temperature causes an increase in intracellular Ca^{2+} ions [31], which can cause even more heat production in the cells.

IV. CONCLUSION

A method for real-time, wireless temperature measurements for cell growth on high-precision thin-film platinum resistors was developed. Highly concentrated, small-volume cell suspensions were seeded on the sensor surface to force the cells to grow only on the sensor. Further, they were cultivated in the incubator. The temperature changes under drug addition (norepinephrine) were detected using a homemade circuit system. According to the results, the human microvascular endothelial cells exhibited a long-lasting temperature rise under norepinephrine stimulation. Many links exist among cells on the sensor surface. The proposed method is helpful to explore interactions among different cells. The in-situ cell temperature measurements using the thermal resistance can be employed to measure the temperature of the original cell state. Therefore, the method is particularly suitable to evaluate the thermogenic ability of cells with other matter or organisms, e.g., materials, drugs, bacteria, and viruses. Because the proposed system can measure the temperature changes of cells in an incubator, the cells are not damaged by a thermometer insertion, close contact with measurement devices, or inappropriate growth conditions. The proposed method can be helpful to diagnose mitochondrial diseases as well as genetic defects of ATP synthase.

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