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DESIGN OF MICROPHYSIOMETER BASED ON MULTIPARAMETER CELL-BASED BIOSENSORS FOR QUICK DRUG ANALYSIS

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Cellular metabolism arouses the changes of substance in extracellular physiological microenvironment, and the metabolic level reflects the physiological state of cells. This paper developed a novel microphysiometer automatic analysis instrument based on multiparameter cell-based biosensors for quick drug analysis. This study included the multiparameter cell-based biosensors, cell culture chamber, drug auto-injection detection and analysis. The analysis instrument was capable of real-time detection for the acidic product and other chemical parameters generated by the cellular metabolism in the micro-volume. Finally, the paper employs human breast cancer cell line MCF-7 and drug experiments to verify the performance of microphysiometer, and study effects of different drugs on cell metabolism. Further, the research explores drug analysis method of the multiparameter microphysiometer. The results showed that the cell-based microphysiometer system provides a utility platform for rapid, long-term and automatic cell physiological environment detection and drug analysis.

Keywords: Microphysiometer; multiparameter cell-based biosensor; extracellular acidification; cancer cells; drug analysis.

1. Introduction

Cellular metabolism is a ubiquitous biological mechanism involved in many significant physiological processes. For a living cell, thousands of biological reactions happen which relate to each other in a complicated network *in vivo*. Most of the biological reactions depend on the energy, and then the cascade reactions are tightly coupled. Finally, the metabolic

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processes provide energy ATP for the normal physiological activities of cells. The cellular metabolism begins with the uptake of oxygen, glucose and other nutrients, then degrades these nutrients to generate the energy ATP and finally secretes the acidic products. On the basis of these processes, the metabolism can be indicated by monitoring the consumption rate of oxygen or glucose, measuring the metabolic heat or detecting the proton production of acidic products.[1,2]

Biosensors sense the molecules with high selectivity based on the molecular specific recognition. The selective active surface combines the bioactive units with electrolyte. And these selective processes ideally depend on the concentration, which change the properties of the sensor surface. Thus the biological information can be translated into the electronic information which can be subsequently converted and amplified into quantitative signals.[3] Plenty of the biosensors have been applied in the field of food safety, environment protection, medicine test and health care. With the development of biosensor, more and more biology, electronics and chemistry technologies have been infused to improve the performance of sensor interface.

Suitable biosensors have been developed to monitor the changes of electrochemical parameters such as chemically sensitive field-effect transistor (CHEMFET), which is a frequently used device to determinate the oxygen and protons. However, field-effect transistor has many disadvantages, including the high cost, a low lifetime, complicated fabrication process and difficult cell culture. Light-addressable potentiometric sensor (LAPS) which was first described by Hafeman *et al.*,[4] is attractive for proton sensibility as well as for simple structure. The first successfully commercialized system with LAPS for determination of extracellular acidification of living cells is the Microphysiometer system, which is released by Molecular Devices Corporation in 1990. Now, we establish a novel cell-based microphysiometer, and engage in applying this platform for the drugs analysis, such as career medicine screening.

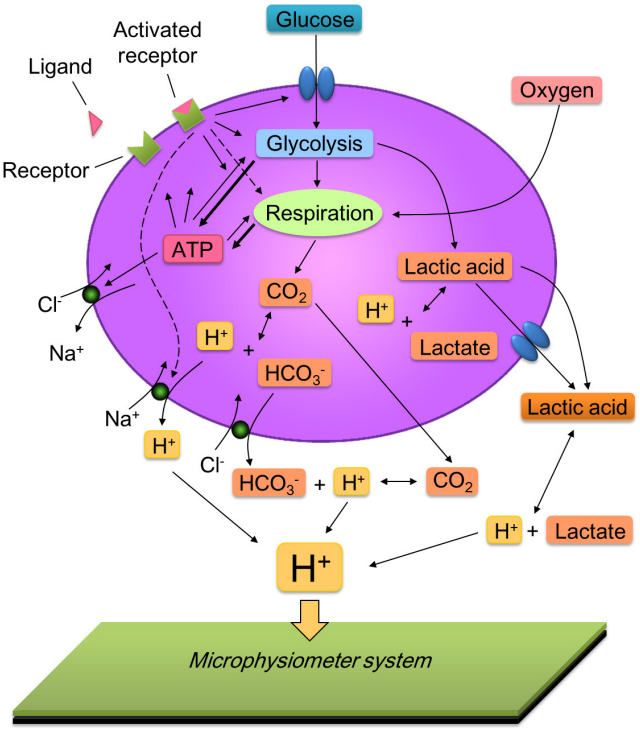


Fig. 1. Schematic diagram of cellular metabolism and relevant physiological processes induced extracellular acidification. With receptor stimulation, the cellular physiological activities will be affected. The corresponding ATP consumption is compensated by the increased uptake and metabolism of glucose, which results in increased secretion of acidic products. The extracellular acidification can be detected by the microphysiometer system.

2. Theory

2.1. *Biological foundation of cellular metabolism*

As the basic physiological feature of living, heterotrophic cells absorb various metabolites, produce the energy and secrete the acidic waste product for growth and development. The metabolic energy is generated by carbon source such as sugars, amino acids and fatty acids. The schematic of cellular metabolism and its relevant physiological process are displayed in Fig. 1. In the normal condition, glucose is taken up by the cells and degraded into energy and acidic products. In the natural aerobic condition, via the glycolysis, citric acid cycle and oxidative phosphorylation, glucose is converted into CO_2 with energy. While in the anaerobic conditions, via the glycolysis, combining lactate dehydrogenase, glucose is converted into lactate with energy.[5] The former reaction is 19 times higher in the terms of ATP generation per glucose molecule — 38 ATP/glucose (aerobic condition) versus 2 ATP/glucose (anaerobic condition). The production rate of acidic product is much less than anaerobic condition with 0.167 H^+/ATP versus 1 H^+/ATP, respectively.

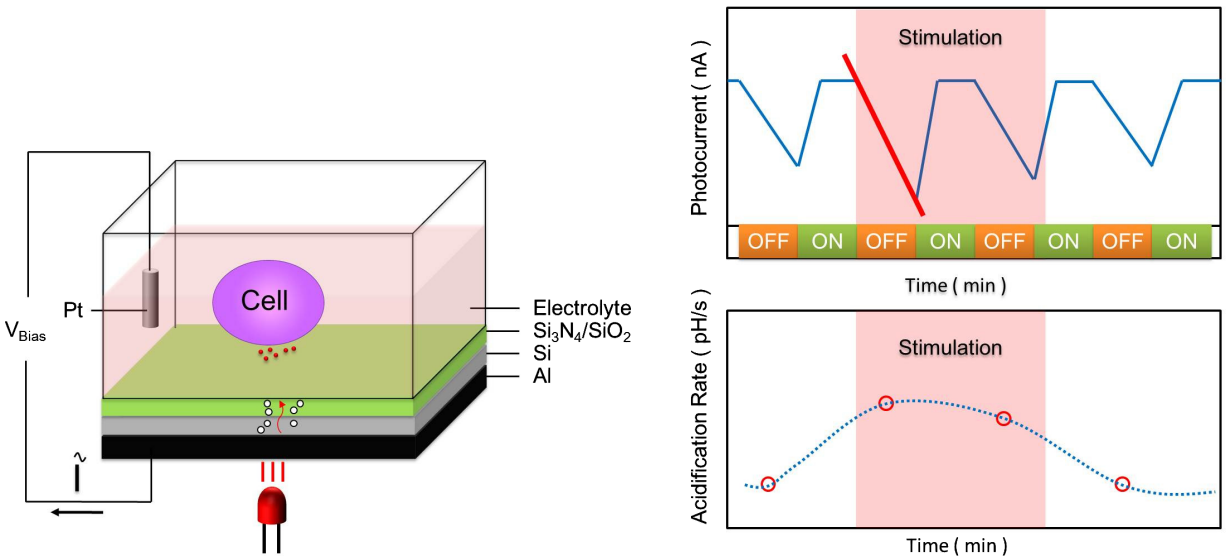
The one mammalian cell in culture medium can produce about 10^8 proton/s. The secretion of acidic metabolites from the cell is performed passively or through other mechanisms.[6] CO_2 and some lactic acid can pass through the lipid membrane via passive diffusion. Lactic acid can also be transported out of the cell by monocarboxilic acid transporter, while bicarbonate can cross the cytoplasmic membrane by some anion transporters, such as the chloride-bicarbonate antiporter. Protons are transported via several H^+-channels and pumps, such as the Na^+/H^+-ATPase.[7] The ion pumps transport the acidic products out of the cell to maintain homeostasis at the cost of energy ATP molecules, which are mainly responsible for increased acidification in the cellular microenvironment. Moreover, some receptors can directly activate the Na^+/H^+ exchanger, and the antiport induces the Na^+ inflow and the H^+ outflow of the cell, which result in the increased acidification of extracellular medium.

CO_2 and lactic acid are the main products from aerobic and anaerobic glucose degradation, which not only can be hydrolyzed into HCO_3^-/H^+ and lactate/H^+ in the cell, respectively, but also can cross the plasma membrane in the form of an unhydrolyzed state and are hydrolyzed out of the cell. Lactate is excreted with facilitated transport by monocarboxylate carriers and anion exchange proteins.[8] Meanwhile HCO_3^- can be transported outside the cell by an antiport. During the hydrolysis process, intracellular and extracellular protons are generated, which have to be transported outside by Na^+/H^+ exchanger. Besides, when the cell receives external stimulation, e.g., exposure to a toxic agent or the activation of a receptor, it can induce the metabolic activity or the ionic equilibrium in the cell, which will lead to the consumption of cellular ATPs. Since ATP hydrolysis is tightly related to the production of acidic metabolites, it can in turn cause changes in extracellular acidification rate. And all these changes reflect that the cell's physiological state can be directly detected by the microphysiometer system.

2.2. *Physical basis for sensing and detecting*

The microphysiometer system is essentially a sensitive pH-meter, and it holds cells in a sensor chamber with the solutions cycle injection system. The core sensor in the chamber is a silicon chip, light-addressable potentiometric sensor (LAPS). This silicon chip is coated with a thin silicon oxynitride as the insulating layer, which can effectively separate the silicon substrate from the electrolyte. The insulating layer interacts with protons in the solution to form a group of silanol (Si−OH) and silamine ($Si-NH_2$). Therefore, the protons in the solution can affect the sensor surface potential.[9] As is shown in Fig. 2(a), if a voltage is applied onto the sensor, an electric field is formed at the silicon−insulator interface. By the modulated infrared light illuminating at the backside of the sensor, a photocurrent is generated.[10] At the atomic level, this photocurrent corresponds to a hole−electron pair creation by radiation absorption from the LED. Since a field potential is formed in the chip, holes and electrons move in opposite directions, resulting in the local current. The photocurrent can outflow from backside aluminum layer.

The amplitude of the photocurrent depends on the applied working potential and surface potential, and surface potential is determined by the pH of the electrolyte on the sensor chip. In the working mode, the most sensitive point of the applied voltage is selected, by which the sensor can reflect the varying pH sensitively. The insulator surface is partially covered by Si-O and few Si-N, both of which can be considered as a function of pH. The surface is neutral at pH about 3.5. Therefore, at physiological pH of cell medium, the surface becomes negatively charged. This feature makes the surface potential pH-dependent. Such dependence is Nernstian and linear from pH 2 to 11.[4] Figure 2(b) shows the schematic recorded during the extracellular acidification experiments in theory, the fluctuations are based on variations reflecting the pH changes. The system applies the working voltage on the sensors. Under the excitation of infrared LED, the sensor generates the photocurrent, which reflects the pH value. As a result, photocurrent can be plotted with time. When the injection pumps are on, the fresh medium washes the solution of micro-environment in the sensor chamber. While injection pumps are off, the cells secreted the acidic metabolites, so the extracellular acidification causes a weakening of the surface potential. The corresponding field potential also diminishes and the photocurrent rises.



(a) (b)

Fig. 2. (a) Schematic diagram of the LAPS detection structure and principle. (b) Recording of the extracellular acidification experiment in theory. Photocurrent is proportional to the pH value. When the pumps are on, waste products are continuously washed away in the flow of medium. When the pumps stop periodically, acidic metabolites accumulate in the chamber, causing pH to drop. Extracellular acidification rate is calculated by the corresponding data sequence in top panel, which reflects the acidification signals determined in the pump-off stage. When stimulation is introduced to the cells, the extracellular acidification rate will have a change compared to that of the basic acidification rate.

Theoretically, with the photocurrent and the pH calibration result of sensor, the derivative of the slope can be calculated as the extracellular acidification rate.

The LAPS surface should be treated by a sensitive membrane to detect other metabolic ions (e.g., Na^+, K^+). LAPS detection is specific to extracellular H^+, since the sensor surface was coated with a thin silicon oxynitride as the insulating layer, which interacts with protons in the solution to form a group of silanol (Si−OH) and silamine (Si−NH_2), and nearly had no response to the Na^+ and K^+. While LAPS was used for Na^+ and K^+ detection, the sensor surface should be coated with the corresponding polyvinyl chloride (PVC) sensitive membrane for Na^+ and K^+. Without PVC sensitive membrane, LAPS showed poor performance for the Na^+ and K^+ detection. Besides, we can also coat the membrane in different region on same sensor surface, with the light-modulation technology to record the different ions response signals simultaneously. Membrane surface-active substances generally contained positively or negatively charged organic ions or complex ions, they formed the corresponding metal ion exchange salts and dispersed in the polyvinyl chloride as matrix system. LAPS sensor with membranes had the selective and sensitive mechanism, which was quantitative performance of analyte's physical and chemical reaction, resulting in potential difference on the membrane surface. The potential difference were not only related to the composition of membrane, but also decided by type and concentration of molecules and ions in the solution. By measuring this potential difference, analyte concentration in the solution can be determined. Generally, we use PVC and chalcogenide glass (ChG) as the membrane material.

In the experiment, cells can survive in the normal state in the absence of any pharmaceutical, which was reflected by the stable release and uptake of H^+, Na^+, K^+. So the ions in extracellular micro-environment will increase or decrease in certain trend. And this trend can be calculated as basal metabolic rates, which were stable. After the stimulation of pharmaceuticals, some receptors, ion-channels or pathways of the cell metabolism were affected, and the cells cannot maintain a normal state. One of the important indications was the release of abnormal ions, so the corresponding metabolic rates will change significantly.

3. Experimental and Methods

3.1. *Microphysiometer sensor unit with flow structure*

The fabrication of LAPS chip was similar to the processes we have reported,[11] so, it has been briefly described. N-type silicon wafers ($\varphi = 4$ inches) with specific resistance of 10−15 Ω·cm were used for LAPS chip. The upper side of the chip was insulated with a layer of 30 nm SiO_2, thermally oxidized at 1000°C. Bulk silicon was grinded to 100 μm thickness to increase the sensitivity. A 1 μm thick aluminum membrane was sputtered on the backside of the wafer to create an ohmic contact. LAPS sensor without surface membrane already had H^+ detection function. And the sensor surface should be dealt with polyvinyl chloride (PVC) membrane before detecting other metabolic ions (e.g., Na^+, K^+), in order to realize the multiparameter detection function of sensor. Microarrayer was applied to coat the tetrahydrofuran (THF) with the mixture of sensitive material. The sensor should be preserved in the dark and undersealed at 4°C for more than 10 h until the THF form sensor sensitive membrane.

Based on the LAPS detection for pH solution, the microphysiometer sensor unit was established to monitor state of physiological micro-environment. We fabricated the micro-structure for recording signals of the cellular metabolism. The schematic of the entire sensor unit is illustrated in Fig. 3(a) and the main features of sensor unit will be detailed in the following paragraphs. The fabrication of micro-chamber played the most important role in the sensor unit. It was the core component in the detection process. Figure 3(b) was the partly structural magnification. The cell metabolic chamber was fabricated by O-ring spacer between two layers of multi-pore polycarbonate membrane. The two multi-pore polycarbonate membranes were fixed at the bottom of the two corresponding sleeves by the nontoxic glue. The spacer was selected by the Polytetrafluoroethylene (PTFE), which was harmless to the cell physiological activity. The thickness of the spacer can be adjusted for the experimental requirements. In our experiment, the 300 μm spacer was selected, thus the chamber volume was less than 8.5 uL in the working state. Upon the detection micro-chamber, the culture exchange chamber was formed by the

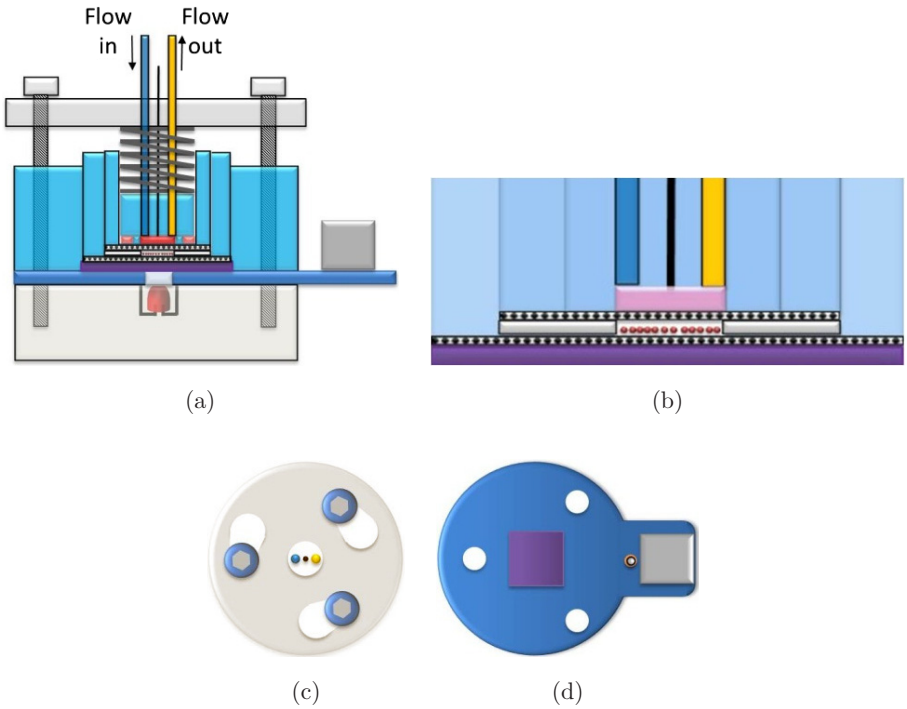


Fig. 3. The sensor unit structure and its special accessories. (a) The schematic of the sensor unit. (b) Cells are entrapped between two polycarbonate membranes of the sleeve. After inserting the sleeves into the fixed base a fluid delivering plunger is placed into the sleeve with the compression spring on the top, creating a micro-flow chamber with the cells. (c) Rotation retainer fixed the sensor units as the cover. (d) LAPS PCB base can be flexibly and easily changed.

plunge and multi-pore polycarbonate membrane at the bottom of the small sleeve. A fluid delivering plunger contained the inflow and outflow channels connecting the culture exchange chamber, which formed the flow system. Besides, the platinum electrode severed as reference electrode in the center of the plunger, whose end has contact with the electrolyte. The O-ring is fixed at the bottom of plunger for better seal by the force of retainer.

The retainer can provide the same force by the rotation design by three screws in the triangle position (see the Fig. 3(c)): the head of screw is slightly bigger than the small circular hole and smaller than the big one. Therefore, the retainer can be fixed in the same position in each experiment, providing nearly the same force with compression spring in the nearly same working length. Thus, the O-ring has the good performance for seal, and the sensor unit can work with the stable state. As is shown in Fig. 3(d), another significant feature is the LAPS PCB base, which is flexibly changed if the sensor chip needs to be cleared before and after the experiments. The LAPS chip is attached at the center of the PCB base by conducting resin, and the standard telephone interface with electrode interface is introduced for better electrical contact. For this separation design, all of these accessories were independent. Based on its replaceable feature, the sensor can easily detect the other metabolic ions (e.g., Na^+, K^+) with corresponding sensitive membranes. Consequently, the whole sensor unit is reusable, which can low the cost of sensors.

3.2. *Culturing of the breast cancer cell line MCF-7 in sensor*

The MCF-7 cells were purchased from Global nonprofit bioresource center (BRC) and research organization ATCC. To culture cells directly on the polycarbonate membrane in the sensor, the sleeve with membrane was laminated by incubating with 5 μg/ml laminin in PBS for 1 h at 37°C. Laminin improves the adhesion of cells onto the membrane.[12,13] About 5×10^4 of harvested MCF-7 cells were resuspended in 1 ml standard culture medium (1% penicillin: Hyclone, cat. SV30010, 10% fetal bovine serum: Gibco, cat. 16000-044, DMEM: Hyclone, cat. SH30022.08), transferred to the culture sensor chamber and kept under standard culture conditions (37°C, 5% CO_2). The membrane was changed by the new one and attached by nontoxic glue for repeated use. And the sleeve was cleared from cells by 1 h tryptic digestion and sonicated for 10 min in 2% ultrasonol solution. After rinsing with distilled water, sensor was disinfected in 70% ethanol for 1 h. Finally, the sensor was washed with sterile PBS.

Moreover, Diff-Quik staining was carried out to verify cellular life in the sensor unit, in order to test the biocompatibility of the whole sensor unit. Diff-Quik is a proprietory brand of a Romanowski stain. The Romanowski group of stains are defined as being the black precipitate formed from the addition of aqueous solutions of methylene blue and eosin, dissolved in methanol. The variants of the Romanowski group differed in the degree of oxidation of the methylene blue stain prior to the precipitation. The stain class was originally designed to incorporate cytoplasmic (pink) staining with nuclear (blue) staining and fixation as a single step for smears and thin films of tissue. The Diff-Quik stain consists of three solutions: Diff-Quik fixative reagent, Diff-Quik solution I (eosinophilic) and Diff-Quik solution II (basophilic). First, the sensor was air-dried, and then DiffQuick fixative (or methanol) was added for 30 s. Subsequently, cells were stained with DiffQuick solution II for 30 s, and counterstain with DiffQuick solution I for 30 s. After the staining, sensor unit was rinsed with water to remove excess stain, and rapidly dehydrated in absolute alcohol. Exposure to alcohol should be as brief as possible to prevent excessive decolorization. Finally, the cells state in the sensor was observed and evaluated by the microscopy.

3.3. *Sensor performance determination and cellular metabolism monitoring*

In order to carry out the cell experiments, we established the flow system by two NE-1000 syringe pumps with the flow rates ranged from 0.001 μL/h to 2120 mL/h, which were purchased from New Era Pump system Inc. They managed to satisfy the requirements of the flow system. In the experiments, they mainly were responsible for the injection of culture medium and drug, respectively. Based on the microfluidic principle, any drug concentration can be formed by controlling the flow rate of each syringe pump. The flow system can pump the solution into the microphysiometer as

the drug supply system. Besides, we also designed the corresponding instrument to realize the function of microphysiometer. The analysis instrument mainly contains the LED driver modules, DA module, amplification modules, PGA module, and AD module, which were responsible for sensor detection.

The microphysiometer sensor unit and the system were displayed in Fig. 4. The sensor unit contained the micro-chamber for the cell culture and the flow structure for the solution exchange. The independent and separate features were convenient for operators to assemble and employ sensor unit. A compression spring supplied the force to form the micro-chamber, in order to maintain the consistency of detection condition. Besides, the standard interface ensured better electrical contact (see Fig. 4(a)).

Microphysiometer system includes the core control and detecting instrument, sensor unit house and calibration box. The core control and detecting instrument managed the whole microphysiometer system, including detection, injection and communication by the corresponding interfaces on the front and back panels. Sensor unit house served as the cell culture greenhouse, which had the constant temperature at 37°C. Therefore, all the experiments can be carried out in the suitable condition.

(a) (b)

(c)

Fig. 4. The microphysiometer sensor unit and the system. (a) Microphysiometer sensor unit for cell culture with flow structure. (b) Microphysiometer system includes the core control and detecting instrument, sensor unit house and calibration box. (c) The whole microphysiometer detection system.

Additionally, the calibration box was designed for the system calibration after the long time usage.

Before the extracellular acidification experiments, the performance of sensors should be determined by $I-V$ curve scanning mode. An $I-V$ curve with good repeatability reflected that the sensor chip had the good stability. After verifying good repeatability, the sensor chip was calibrated by the pH standard solution (5, 6, 7, 8). These solutions were continually pumped into the microvolume of sensor chamber in turn under the same condition with cell experiments. And each pH was recorded for about 5 min to calculate the mean of its raw data. Thus, four means were obtained for plotting the calibration curve about pH versus photocurrent. Therefore, the actual photocurrent can be easily converted into corresponding pH. All of these will be detailed in the result and discussion section.

Extracellular acidification experiments followed sensor performance determination. In the microphysiometer sensor unit, the breast cancer cell line MCF-7 were kept alive for hours or even days due to the suitable cell culture condition. About 50 k/mL cells were cultivated in a micro-volume sensor chamber formed by the two multi-pore polycarbonate membrane, where the cells were entrapped in the culture space, while the ion and other acidic waste can pass through the membrane and exchange with the ion in the flow chamber freely. In the CO_2 incubator, the cells were cultured directly on the membrane. Then, a sleeve with MCF-7 cell lines was transferred from incubator into the sensor unit. One syringe pump injected the culture medium and drug through a heater into the flow chamber. The culture or drug medium used for refreshing the micro-volume sensor chamber contained only 1 mM phosphate as buffer without bicarbonate. However, an appropriate amount of NaCl was added to the medium to keep the osmotic balance state. PBS with low buffer capacity ensures a high sensitivity of the system allowing it to detect smaller pH changes. The pump was switched off for about 10 min to observe the extracellular acidification, and then switched on for about 5 min for refresh medium in the detection region. This circle was carried out for many times to obtain the basal acidification of cells in the steady state condition. Subsequently, the syringe pump was controlled to transport the drug into the sensor chamber. The extracellular acidification was also recorded in the presence of 1 μg/mL glucose and 1 μg/mL clostridium difficile toxin B. After sensor response signals successfully recorded, the acidification rate can be converted by these raw data. Besides, the acidification rates were compared in the absence and presence of drug. Finally, the following drug analysis will be performed on this drug valuation platform.

4. Results and Discussion

4.1. *Performance of sensors for acidification detection*

The $I-V$ curve was the most significant index to reflect the basic characteristics of the LAPS sensor. The $I-V$ curve represented the relationship between photocurrent generated by sensor and the bias voltage applied on the sensor. Therefore, the $I-V$ scanning mode was applied at first. All the results from calibration experiments were illustrated in Fig. 5. The $I-V$ curve results presented a good linearity and repeatability, which indicated that the sensor had a steady state with less drift about 2% in the linear working region in long-time detection (see Fig. 5(a)). Then the most sensitive point was calculated as working voltage in the long-time constant voltage mode.[14] Based on the result, the calibration experiments were carried out to determine the relationship between pH and photocurrent. Figure 5(b) displayed solution with pH 5, 6, 7 and 8 in the long-time mode under the working voltage. The mean photocurrent under each pH was derived to employ linear fit to deduce conversion formula. The fit results were shown in Fig. 5(c) with a linearity coefficient of $R^2 = 0.9995$. The sensor showed a good sensitivity in the considered range. Thus, when photocurrent shifts, the corresponding pH changes can be determined. By conversion of the photocurrent and pH, the baseline shift standard error was about 10 nA. Therefore, the detection resolution of sensor was about 50 mpH, which satisfy the requirements of cellular metabolic experiments. In the conventional detection, the sensor sensitivity was determined by $I-V$ scanning mode, and our sensor was about 50 mV/pH. In our experiment, because of the quick analysis requirement, we applied the sensors by the constant working voltage mode. So the sensor detection sensitivity was about 195 nA/pH, which can be calculated by the calibration results. Moreover, we carried out the Na^+ and K^+ calibration experiments, the results were shown in

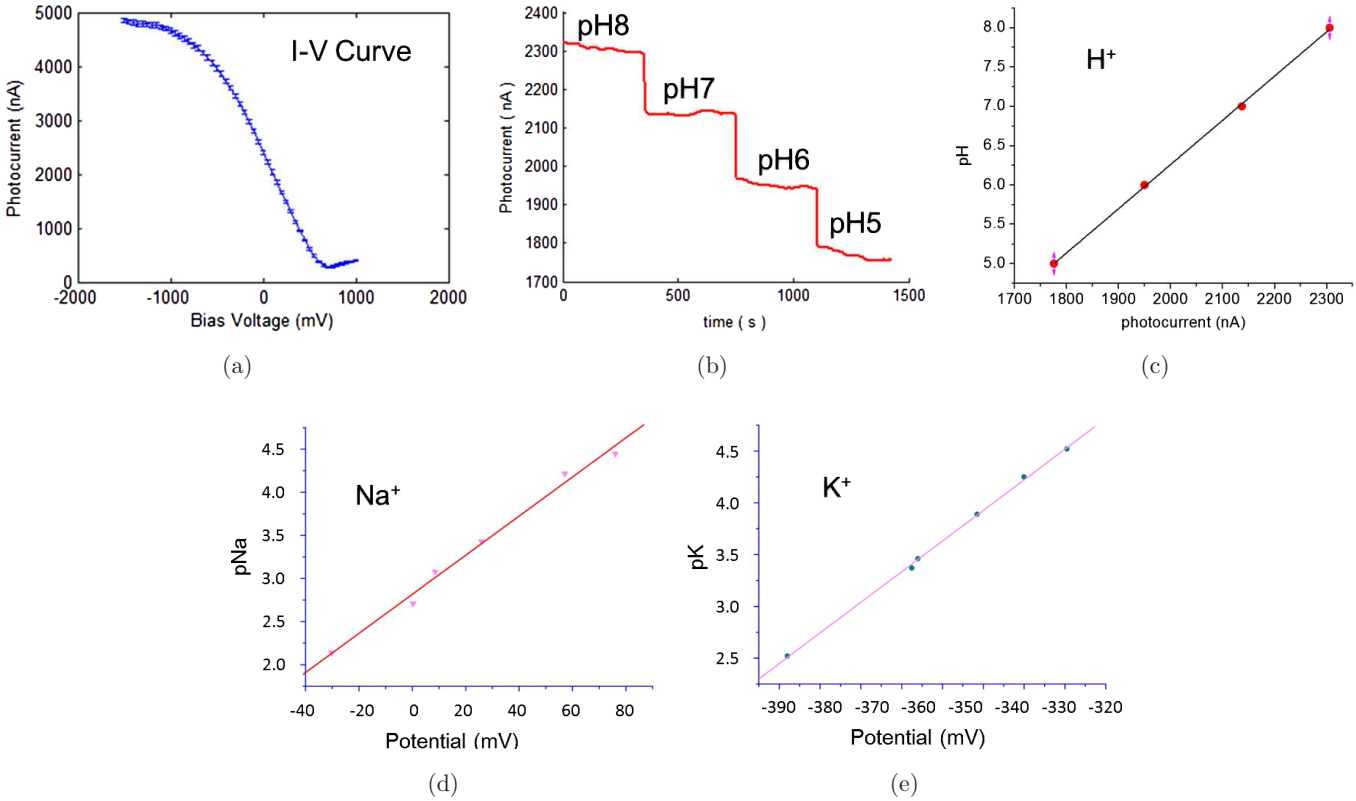


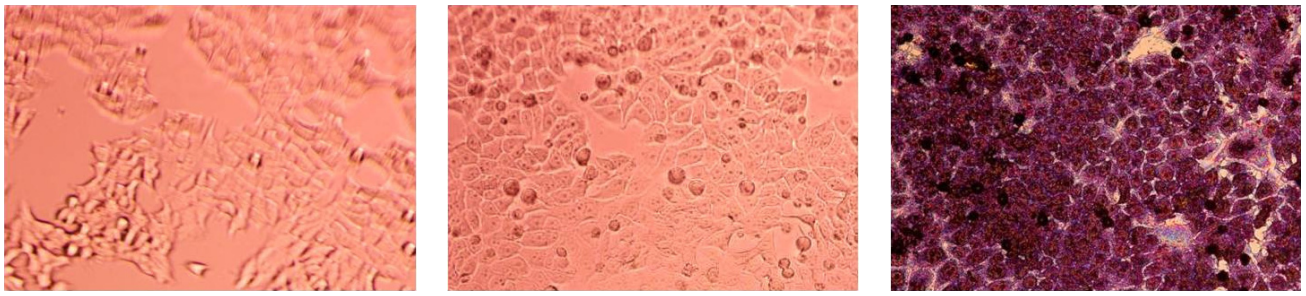
Fig. 5. The test and calibration results of LAPS sensor performance. (a) Linearity and repeatability test result of sensor in the $I-V$ curve scanning mode. (b) Typical calibration results between the pH and photocurrent of sensor in the long-time constant voltage mode. (c) Conversion relationship between the pH and photocurrent of sensor by linear fit. (d) Conversion relationship between the pNa and surface potential of sensor by linear fit. (e) Conversion relationship between the pK and surface potential of sensor by linear fit.

Figs. 5(d) and 5(e). From the results, we can conclude that LAPS also had a good linear response to Na^+ and K^+.

4.2. *Cell culture in the sensor chamber*

In the experiments, the MCF-7 cells were cultured on the multi-pore polycarbonate membranes in the sleeve of the sensor unit, for the two reasons. One reason was to determine the biocompatibility of multi-pore polycarbonate membranes. Biocompatibility was the most important factor in a series of experiments. If the cells cannot survive on the membranes due to weak biocompatibility, they cannot maintain normal shape, and cellular metabolism will not be in the normal state, and the results cannot be persuasive. The other reason was to determine the suitable number of cultured cells. An appropriate number of the cells will also affect the results. If the number of cells was small, they can cover the whole membrane surface. The extracellular acidification signals cannot be reflected obviously, and we may miss significant information. While the number of cell was too large, they also cannot have a normal state due to lack of space and nutritions. Therefore, the biocompatibility of the membrane and sensor and the appropriate number of the cells were key factors in the cellular acidification experiment.

The MCF-7 culture results were displayed in Fig. 6. The 50 k/ml Cells were relatively few and scattered in 24 h later (see Fig. 6(a)), while it became dense 48 h later (see Fig. 6(b)). After the Diff-Quik staining, we can conclude that the MCF-7 developed well with the normal state, and the 50 k/ml MCF-7 closed to the 95% fusion level on the culture membrane surface (Fig. 6(c)). Its fusion level will satisfy the detection requirement. Taking the quick development of the cancer cells and sufficient supply of nutrition in the micro-environment into consideration, the number of 50 k/ml was selected in extracellular acidification experiments.



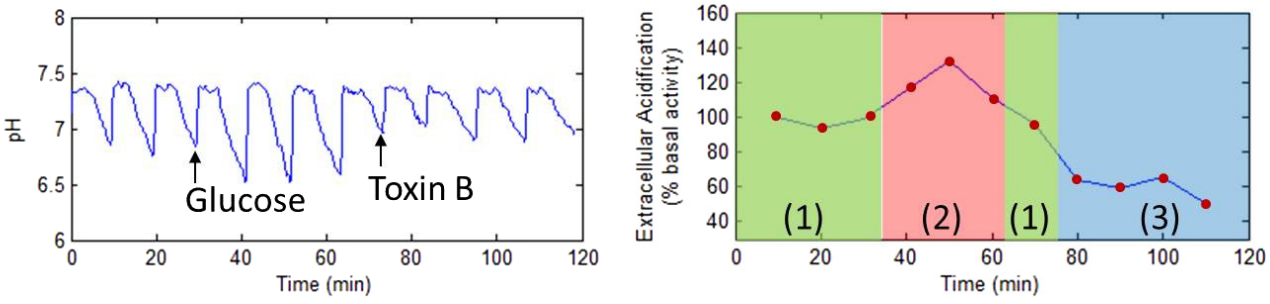
(a) (b) (c)

Fig. 6. The human breast cancer cell MCF-7 cultured on the multi-pore polycarbonate membranes in the sleeve of the sensor unit. Cell number was about 50 k/ml, respectively. Cells state in (a) 24 h later, (b) in 48 h later, (c) Diff-Quik detection in 48 h.

4.3. *Metabolic activities monitoring for drug analysis*

In our experiment, the metabolic activities of MCF-7 cells were monitored by the secretion of the cellular acidic metabolites. The extracellular acidification rate was one of the most significant parameters that indicated cellular metabolic rate, therefore, we carried out the cellular experiments on monitoring the extracellular acidification to determine the performance of the microphysiometer. First, the extracellular acidification rate was recorded in the presence of cell culture medium alone as basal extracellular acidification rate. Subsequently, glucose and clostridium difficile toxin B were injected into the sensor unit to observe the change of extracellular acidification rate, respectively. Figure 7 displayed the cell experiments in the stage of blank (1) glucose (2) clostridium difficile toxin B (3). In the absence of drug, the cells secreted the acidic metabolites in the native state. Meanwhile, the injected glucose induced the extracellular acidification rate, resulting in a sharp increase in the MCF-7 cells, and the maximum about 138% was reached after the 40 min, which recovered to the basal level after washing by the PBS. Next, the clostridium difficile toxin B was injected into the culture medium, which induced the extracellular acidification rate decrease of 40% of the basal after 70 min.

In this investigation, the breast cancer cell line MCF-7 was used in the drug experiments. The MCF-7 cells can live with the normal state in absence of stimulation, which was reflected by the stable release and uptake of H^+. The H^+ secretion caused the pH decrease in extracellular microenvironment with the syringe pump on. While the syringe pump was off, the acidic waste products were taken out by refreshing the culture medium, and the pH recovered to the original state with increase of corresponding photocurrent. The extracellular acidification rate was calculated as one of important indication parameters for the cell physiological state. In the culture buffer without pharmaceuticals or other stimuli, the cellular H^+ released in the similar rate and presented the same extracellular acidification level, which was defined as the basal metabolic rate and reflect



(a) (b)

Fig. 7. The typical drug experiment results of cellular metabolism. (a) Original data shows the change of pH corresponding to the photocurrent. (b) The extracellular acidification rate under the blank (1), Glucose (2) and Clostridium difficile toxin B (3).

stable cell metabolism. After the glucose was added into the cell culture chamber, the pH in the microenvironment dropped more sharply and extracellular acidification rose significantly for the more uptake of glucose and enhancement of the respiration. For the drug experiment, clostridium difficile toxin B was added into the culture buffer, and then the extracellular acidification rate gradually decreased to a lower level for death of some cells. Toxin B was frequently cytotoxic and killed cells by altering the apical membrane permeability of the epithelial cells, so the toxin B induced the extracellular acidification rate decrease.

Glucose was the necessary nutrition and supplied the main energy ATP in the cell normal life. With the glucose increase, intake of glucose was sharply raised by MCF-7 cells, which increase the generation of the protons under aerobic or anaerobic condition. Therefore, after the cells absorbed more glucose, metabolic activities were enhanced in the form of an increase in the extracellular acidification rate. Meanwhile clostridium difficile toxin B was a toxin generated by *Clostridium difficile* bacteria. It is usually described as an enterotoxin, but it also has some activity as a cytotoxin,[15] toxin B were chromosomally encoded exotoxins that are produced and secreted from several bacterial organisms. They were often heat-stable, and were of low molecular weight and water-soluble. Enterotoxins were frequently cytotoxic and kill cells by altering the apical membrane permeability of the epithelial cells. The action of enterotoxins leads to increased chloride ion permeability of the apical membrane of cells. These membrane pores are activated either by increased cAMP level or by increased calcium ion concentration intracellularly. Therefore, the toxin B affected the normal metabolic activities of the MCF-7, which induced a weak extracellular acidification rate.

The instrument can realize the long-term detection, and experiments on cell metabolism can be carried out for at least 3 h, depending on the stable cell state, which was due to several reasons. First, the recording was non-invasive, so the cellular structure and function was undamaged and the cells survived longer in the experiment. Cell flow and culture structure of sensor unit was another reason for long-term detection. The flow structure ensured that the culture buffer and other necessary nutrients were pumped into the cell culture chamber and the waste products were taken out continually duration the experiments. Besides, two layers of multi-pore polycarbonate membrane formed the cell culture chamber, which can trap the cells tightly and healthily without being taken away by the perfusion buffer, and thus whole cells culture sleeve can also be taken out of the sensor unit and recovered in the cell incubator after some experiments, so the cells can live in the normal state for a long time. Therefore, after the cells state was preserved, the instrument can satisfy the requirements of long-term experiments.

5. Conclusion

We were involved in developing a novel cell-based microphysiometer, by improving the analytical instrument from cell-based biosensor, detecting instrument and drug experiments. We designed a new micro-chamber structure of a sensor unit, and realized the monitoring the extracellular physiological micro-environment by light-addressable potentiometric sensor. By employing human breast cancer cell line MCF-7 and studying effects of different drugs on cell metabolism, the performance of microphysiometer was verified for detecting extracellular acidification rate. Therefore, the novel cell-based microphysiometer system provides a utility platform for rapid, long-term and automatic cell physiological environment detection and drug analysis.

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