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A LAPS array with low cross-talk for non-invasive measurement of cellular metabolism

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ABSTRACT

Cellular metabolism is ubiquitous biological mechanism involved in many significant physiological processes, and most of the biological cascade reactions are tightly coupled. Non-invasive measurement of cellular metabolism is important to study the metabolism mechanism and drug effect in a long-term detection. In this study, the two processes of heavy doping and thick oxidation fabricated a new light-addressable potentiometric sensor array, which was applied to detect the cellular metabolism induced pH change. The performance of the LAPS was tested by the basic characteristic experiments and renal cell experiments. The result showed that the stability and pH resolution of LAPS array were greatly improved. With the development of the sensor design and fabrication, the LAPS will be a utility tool in the field of biological metabolism.

1. Introduction

Cellular metabolism is the set of reactions that happen in the cells of living organisms to sustain basic life support. These processes allow cells to grow and reproduce, maintain their structures, and respond to fluctuation of physiological microenvironments [1]. Energy metabolism is one of the most important cellular metabolisms, which provides the energy for living cells. Cells begin with the uptake of the nutrition and oxygen, and generate the acidic metabolites by processes of glycolysis and respiration [2–5]. In order to maintain the steady intracellular environment, cells excrete these acidic metabolites into the microenvironment, which results in the extracellular acidification [6–8].

The former researches have reported many cell assays for effective drug analysis [9–12]. The traditional study of the ion channels and metabolism is patch clamp, which is a standard method to study cellular electrophysiology [13]. The first measurements of cell pH were performed using microelectrodes [14]. However, these studies caused considerable damage to the cells or whole cell layers in the invasive way. The respective extracellular and intracellular pH of cells is also monitored in vitro by non-invasive methods, such as magnetic resonance spectroscopy [15]. But magnetic resonance techniques require a great deal of expensive equipment and they are not suitable for continuous, long-term data acquisition and monitoring of living cells. Due to these reasons, light-addressable potentiometric sensor (LAPS) is introduced to monitor the extracellular pH.

LAPS is a semiconductor device, which is similar to the ion selective field effect transistor (ISFET) [16,17]. The significant features of the LAPS are complete flatness and the simple fabrication. Comparing to ISFET, LAPS sensing surface is free of any metal contact and ensures a very good flatness. It implies that the combination of the sensor and chambers is very easy, where flowing solutions contact the sensing surface. The manufacturing process of LAPS is simple and LAPS chips present a much lower cost than that of ISFETs [18–20]. Therefore, LAPS was widely applied in the electrochemical detection [21–23]. Many endeavors have been made to improve the pH sensitivity of LAPS [24,25]. However, the pH resolution was more important for the detection of extracellular acidification. The previous highest LAPS resolution was about 0.5×10^{-3} pH U based on the silicon oxynitride insulator layer [26]. The pH resolution played an important role in the extracellular metabolism detection. With higher pH resolution and better stability, LAPS was sensitive to smaller pH change without being disturbed by the cross-talk effect.

LAPS has a light-addressable characteristic that the sensitivity of illuminated region was enabled. Owing to this feature, LAPS has an advantage for detecting the arbitrary position on the sensor. However, cross-talk will be introduced due to scattering of light and absorption of photons in undesirable regions. In order to improve the sensor stability, some materials (e.g. polyimide and photoresists) were covered onto the undesirable regions to decrease the

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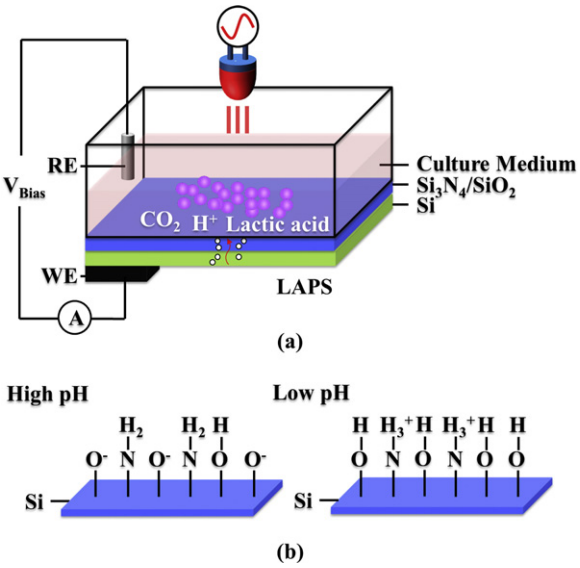

Fig. 1. The LAPS detection principle for the extracellular acidification. (a) The schematic of LAPS detection principle. (b) The surface state of LAPS under the high pH and Low pH condition.

photons absorption on them [27,28], but they may degrade in the water solution or have the toxicity for long-term cell culture.

In this study, a light-addressable potentiometric array was introduced for mornitoring the cellular metabolism in a non-invasive way. The stability and pH resolution of LAPS array were higher than the normal LAPS by inhibiting the cross-talk efficiently. Moreover, the LAPS array performance was determined by basic characteristic test and extracellular acidification experiments of renal cells. All the details will be described in the following sections.

2. Theory

2.1. Cellular metabolism basis

For living mammal cells, most of the biological reactions are maintained by energy metabolism activity, and the cascade reactions tightly occur with the sufficient energy supply. Cells initially absorb glucose by a transport protein. Subsequently, glucose is metabolized to CO_2 by the respiration and to lactic acid by glycolysis. Both weak acids are dissociated to generate H^+ at the cellular physiological pH. The CO_2 can also diffuse across the membrane in passive way, while the lactic acid can transport out of cell by monocarboxylic acid transporter. And H^+ Pathways for H^+ efflux include the Na^+–H^+ exchanger as well as various H^+ channels and pumps [29]. All these acidic products pass through the plasma membrane in facilitated and unfacilitated ways and accumulate in the extracellular microenvironment, which results in the extracellular acidification [26,30,31]. Based on this principal cellular mechanism, the metabolism can be indicated by monitoring acidic products. Significantly, the microenvironment pH of living cells acts as an important indicator for a number of cellular functions. If the energy metabolism is disturbed, the net result is the fluctuation of extracellular acidification.

2.2. LAPS working principle

Light addressable potentiometric sensor (LAPS) is a semiconductor device that has pH-sensitive surface with an electrolyte–insulator–semiconductor (EIS) structure. As shown in Fig. 1(a), LAPS consists of $Si/SiO_2/Si_3N_4$. When a DC bias voltage is applied between the working electrode (WE) and reference electrode (RE), a depletion layer is formed at the insulator–semiconductor interface. By illuminating with a modulated infrared LED, an AC photocurrent with the same modulated frequency generates in the depletion layer of LAPS. The amplitude of AC photocurrent is sensitive to the surface potential. And thus the electrochemical event-induced surface potential change can be monitored by LAPS. For pH measurement (Fig. 2(b)), LAPS surface contains primarily silanol group with a small percentage of silamine group. The silanol group exists in the form of SiO^- or neutrally charged Si—OH. The silamine group exists in the form of Si–NH_2 or Si–NH_3^+ [16,17]. Therefore, the surface potential is more negative at high pH than at low pH.

3. Experimental and methods

3.1. LAPS array fabrication

P-type silicon wafers (500 μm thick and 4 in. in diameter) were selected for LAPS chip with specific resistance of 10 Ω cm and orientation index of crystal orientation ⟨1 0 0⟩. The fabrication process of this LAPS array was slightly different from the normal one (Fig. 2). After RCA cleaning process, the wafer was thermally oxidized at 1000 °C to form a SiO_2 layer. And then a SiO_2 layer was partially removed through HF etching after photolithography. Thermal diffusion doping was then carried out. There were two processes in doping process. Firstly, boride is deposited to form P^+ region as dopant by the fast diffusion. In the following process, the high temperature drive-in is applied to give sufficient surface doping concentration. During the high-temperature drive-in step, a 600 nm silicon layer was formed on the doping areas. After the doping process, the SiO_2 on the sensitive regions defined by mask was removed through HF etching. Subsequently, the normal LAPS fabrication processes were carried out. A 50 nm SiO_2 layer was formed on sensitive regions, which are thermally oxidized at 1000 °C. And then a 10 nm Si_3N_4 layer was deposited by LPCVD as a sensitive and protective layer on the surface of SiO_2 layer. Thus, the detection regions were formed at desired positions. Finally, an aluminum layer about 300 nm in thickness was evaporated on the backside of wafer to form an ohmic contact.

The width of depletion layer when the charge space of LAPS works at depletion is

$$x_d = \frac{2(V_{BIAS} - V_M)/q}{\left[(d_0 N_A/e_0 e_{ro})^2 + 2(V_{BIAS} - V_M)N_A/e_0 e_{rs} q\right]^{1/2} + (d_0 N_A/e_0 e_{ro})} \quad (1)$$

where x_d presents the width of depletion layer, e_0, e_{ro}, and e_{rs} are dielectric constants of vacuum, silicon and silicon dioxide, q is the charge of an electron, V_{BIAS} is bias on electrode, V_M is the voltage on solid/liquor interface, V_M is constant when state of the solution is invariable, including pH of the solution, concentration and characteristics of the respective ions. d_0 is the thickness of oxide layer, N_A presents the doping concentration in P-type silicon substrate. From the equation, it presents that the doping concentration N_A and the thickness of oxide layer d_0 can lead the decrease of depletion layer width x_d [32].

P^+ heavy doping and the thick oxide layer were two significant factors for LAPS array, as shown in Fig. 3. Based on P^+ heavy doping and thick oxidation layer, the sensitive regions were divided into small independent detection sites, by cutting off the depletion layer of neighbor detection site. Also, heavy doping was also formed at backside of wafer to form better ohmic contact with the aluminum layer, which served as the working electrode (WE).