A High Sensitivity Biosensor for Cellular Metabolism Monitoring Based on Improved Light-addressable Potentiometric Sensor and Constant Voltage Detection

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Abstract:

A high sensitivity biosensor based on light-addressable potentiometric sensor (LAPS) was established for monitoring the cellular metabolism. Constant voltage detection mode was introduced to replace the conventional constant current mode. Moreover, the sensitivity of LAPS surface potential was improved by reducing the thickness of the semiconductor substrate. By these means, the sensitivity of cellular metabolism monitoring was 1104 nA/pH in the most of extracellular microenvironment (pH6-pH8). Cellular metabolism experiments were carried out to determine the sensor performance. The result showed that characteristics of LAPS have a better performance to sensor the change of cellular metabolism rapidly and sensitively in real time detection. By the improved LAPS and the novel detection mode, the high sensitivity biosensor is promising to improve to study the mechanism of cellular metabolism.

Key words: high sensitivity biosensor, light-addressable potentiometric sensor (LAPS), constant voltage detection, cellular metabolism

Introduction

Cellular metabolism is a common 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 processes provide energy ATP for the normal physiological activities of cells. (See Fig.1(A)) 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. In aerobic condition, via the glycolysis, citric acid cycle and oxidative phosphorylation, glucose is converted into CO_2 with energy. While in anaerobic conditions, via the glycolysis, combining lactate dehydrogenase, glucose is converted into lactate with energy. With receptor-ligand interaction, the cellular physiological activities will be affected.
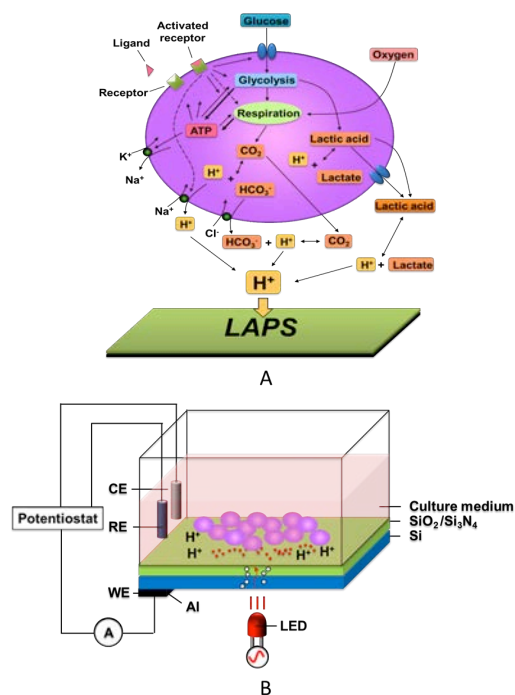

Fig.1. (A) Schematic of various cellular metabolism and processes induced extracellular acidification (B) H^+ concentration detection principle by LAPS.

The corresponding ATP consumption is compensated by the increased uptake and metabolism of glucose, which results in increase of acidic products. The extracellular acidification induced H^+ concentration can be detected by LAPS in the real time.[1] The light-addressable potentiometric sensor (LAPS) is essentially a sensitive pH-meter with an electrolyte/insulator/semiconductor (EIS) structure [2, 3]. (See Fig.1(B)) The insulator consists of SiO_2 and Si_3N_4, which can effectively separate the silicon substrate from the electrolyte. The insulating layer interacts with H^+ in the solution to form a group of silanol (Si-OH) and silamine (Si-NH2). The H^+ concentration in the solution can affect the sensor surface potential [4]. If a bias voltage is applied onto the reference electrode of sensor, a depletion layer forms at the silicon-insulator interface. By illuminating the frequency-modulated infrared light at the backside of the sensor, a photocurrent with same frequency is generated. This ac photocurrent corresponds to a hole-electron pair creation by radiation absorption from the LED. The amplitude of photocurrent is a function of surface potential and can be detected from backside aluminum layer by the instrument [5, 6].

In this study, a high sensitivity biosensor based on LAPS was established for monitoring the cellular metabolism. Constant voltage detection mode was introduced to replace the conventional constant current mode. The sensitivity of LAPS surface potential was improved by reducing the thickness of the semiconductor substrate. The performance of biosensor will be determined by the basic characteristic experiments and the cellular experiments.

Constant Voltage Detection

Current-Voltage characteristic curve plays an important role in indicating the pH sensitivity of LAPS. A series of the sigmoid curve is similar with a pH spectrum which reflects the relationship between the induced photocurrent and the bias voltage applied to sensor LAPS under the different pH. The sigmoid curves have three regions as shown in Fig. 2. In cutoff region, there is no photocurrent. In saturation region, the photocurrent is close to maximum. In working region, the photocurrent increases as bias voltage decreases (n-type LAPS). Conventionally, the method of LAPS pH detection was by constant current mode, which monitors the change of inflection point voltage ($\triangle$X). And C-V curve shifts toward positive potentials as the solution pH increases. The conversion of pH and inflection point voltage

can be determined by calibration. The constant current mode was commonly used to detect the H^+ concentration corresponding to the sensitivity of Nernst equation directly [7, 8]. However, the photocurrent amplification of LAPS was ignored. If the LAPS has the large amplification factor of k (nA/mV) in the linear working region, the constant voltage mode will be more suitable than constant current mode to detect the H^+ concentration in the range of normal extracellular pH.
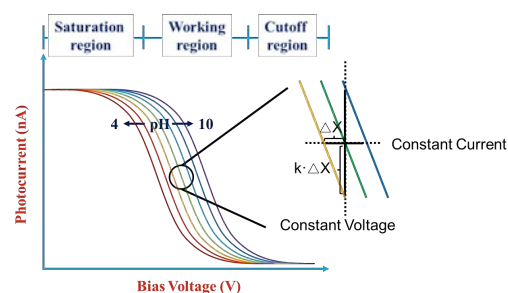

Fig.2. Typical C-V curves of LAPS sensor under the different pH and the monitoring parameters under constant current mode and the constant voltage mode.

Sensor Fabrication

LAPS device usually consists of the heterostructure of $Si/SiO_2/Si_3N_4$. N-type silicon wafers (500 um thick and 4 inches in diameter) were selected for LAPS chip with specific resistance of 10 Ω·cm and orientation index of crystal orientation <1 0 0>. As illustrated in Fig.3, The silicon chip surface was insulated with a layer of 50 nm SiO_2 by thermally oxidation at 1000 ℃. Then a thin layer of 50 nm Si_3N_4 was deposited by LPCVD as a sensitive and protective layer on the upper side of SiO_2. Significantly, the bulk silicon was grinded to 100 um to increase the sensitivity in our chip design. An aluminum membrane, about 300nm in thickness, was finally evaporated on the backside of the silicon chip to form an ohm contact.
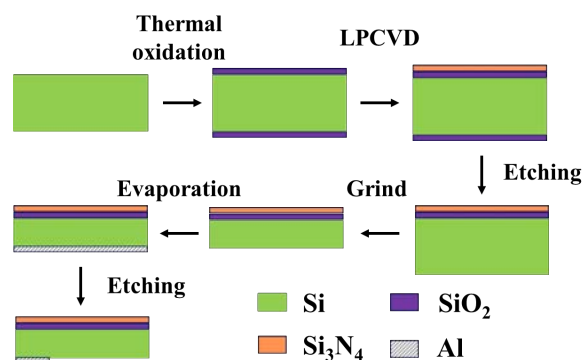

Fig.3. Fabrication processes of the improved LAPS.

LAPS performance experiment

Before the extracellular acidification experiments, the performance of sensors should be determined by C-V curve scanning and the sensor calibration. C-V curve (Fig.4) showed that the 100 um thick LAPS had a significant large amplification factor k than that of 500 um thick LAPS.

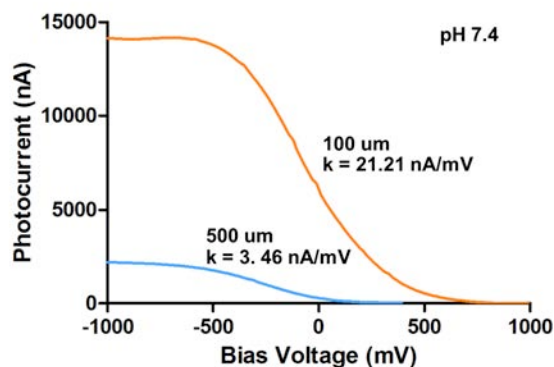Fig.4.C-V curve of 100 um and 500 um thick LAPS at the pH 7.4.

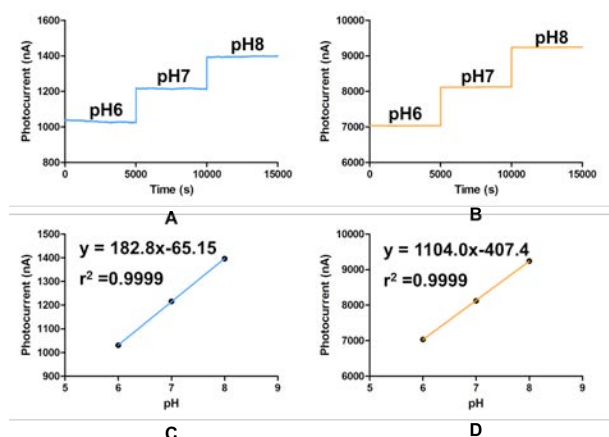
Fig.5. Calibration of 100 um and 500 um LAPS from pH6 to pH8 under the constant voltage detection mode.

After C-V curve scanning, the sensor chip was calibrated from pH6 to pH8. Each pH was recorded for about 5000 s to calculate the mean of its raw data Fig.5(A) and (B). From the calibration curve in the Fig.5(C) and (D), the sensitivity of improved LAPS was about 1104 nA/mV versus 182.8 nA/mV of original LAPS. The calibration results also verified that the 100 um LAPS has a high sensitivity in the considered range, which can satisfy the requirements of cellular metabolic experiments.

Human breast cell line culture

The MCF-7 culture results were displayed in the Fig. 6. The 50k/ml Cells were dense in 48 hours later (See Fig. 6(A)). After the DiffQuik dye, we can conclude that the MCF-7 developed well

with the normal state in 48 hours culture, and the 50k/ml MCF-7 closed to the 95% fusion level in the sensor (Fig. 6(B)) which satisfied the detection requirement.
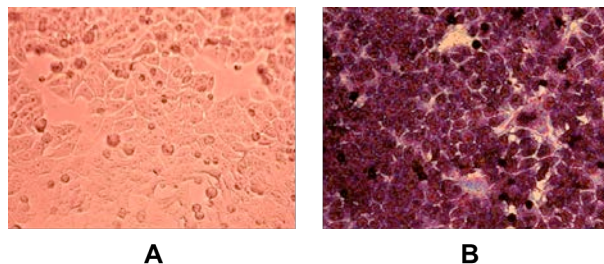

Fig.6. Human breast cancer cell MCF-7 cultured in 48 hours later (A) and DiffQuik dye (B)

MCF-7 Extracellular acidification experiment

Finally, MCF-7 Extracellular acidification experiments were carried out to test the performance of the improve LAPS and constant detection mode. The metabolic activities of MCF-7 cells were monitored by the secretion of the cellular acidic metabolites. From the results in Fig.7, we can see that the extracellular acidification can be detected in a relative short time in the experiment.
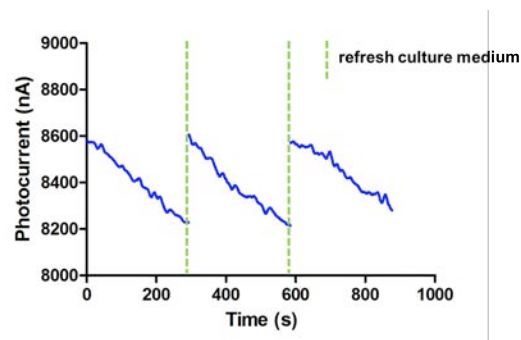

Fig.7.Typical result of extracellular acidification experiment based on MCF-7 cells.

Conclusion

We engaged in developing a high sensitivity biosensor by introducing the constant current detection mode and improving the structure of the light-addressable potentiometric sensor. By employing human breast cancer cell line MCF-7, the performance of high sensitivity biosensor was proved for detecting extracellular acidification rate.

Acknowledgements

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