

# A Novel Design of Cell-based Biosensors for Detecting Cell Acidification

Tao Liang<sup>1</sup>, Qian Wu<sup>1</sup>, Jiawei Tu<sup>1</sup>, Ying Gan<sup>1</sup>, Zhen Qin<sup>1</sup>, Hao Wan<sup>1</sup>, Ping Wang<sup>1</sup>

<sup>1</sup> Biosensor National Special Lab, Key Lab for Biomedical Engineering of Ministry of Education, Department of Biomedical Engineering, Zhejiang University, Hangzhou, Zhejiang, China  
cnpwang@zju.edu.cn

## Abstract:

Cell metabolism is a common biological mechanism in living cells. Acidification plays an important role in cell metabolism, thus the extracellular pH change is used to indicate the vitality of cells. For extracellular detection of cell metabolic substances, light addressable potentiometric sensor (LAPS) has many advantages, such as high sensitivity, easy encapsulation, and perfect to make microchambers for cell experiments. LAPS is a spatially resolved biochemical sensor based on field-effect. Characteristic test experiments and cell metabolism experiments were carried out to determine the performance of LAPS by monitoring the pH change of human colon adenocarcinoma cells Caco-2. The pH sensitivity is 53.69mV/pH, the pH of Caco-2 cells changes from 6.5 to 5.2 and the rate of pH change decreases after 30min. The results showed that the system performed well in real-time detection of cell acidification.

**Key words:** LAPS, extracellular detection, cell metabolism, acidification, pH.

## Introduction

Cellular metabolism is composed of biological reactions, most of which can be indicated by the production of acidic products. The process of metabolism can be reflected by the pH change in the extracellular microenvironment, thereby reflecting the vitality of cells [1]. For example, the cell acidification of tumor cells was higher than that of the normal population, which can be attributed to a transformation-induced alteration of the glycolytic metabolism and glucose transport. Existing detection methods usually cost much and complicated to operate. Light-addressable potentiometric sensor (LAPS) was reported as a suitable tool because of the high sensitivity of pH and non-invasive detection [2]. In this work, characteristic test experiments and Caco-2 cells metabolism experiments were carried out, the details are described in the following text.

## Principle

LAPS is a spatially resolved biochemical sensor based on field-effect, Fig.1a shows the structure of LAPS. Depletion layer forms when a DC bias voltage is applied. The characteristic I-V curve is obtained due to the formation of photocurrent in depletion layer since the chip is illuminated by modulated light. Sensitive

material on the surface of chip allows H<sup>+</sup> to be adsorbed specifically (Fig.1b), thereby affecting the surface potential [3].

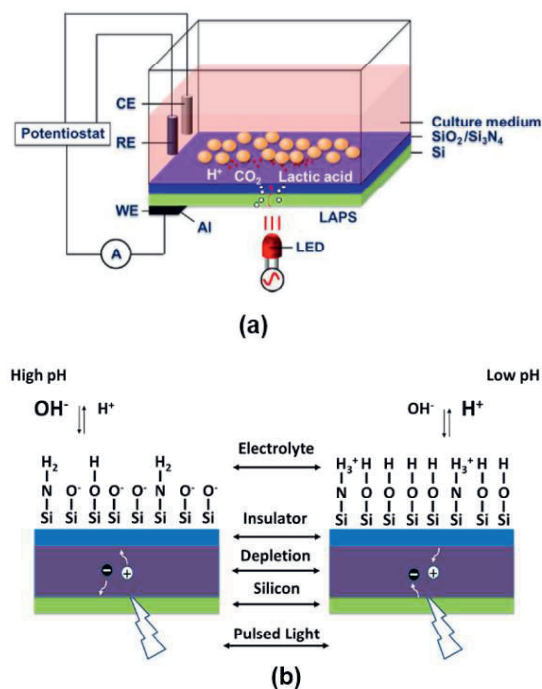


Fig.1. (a) Schematic of LAPS system; (b) Basic principles of LAPS for pH detection.

### Characteristic Test Experiment

In this experiment, n-type Si (100) was used as the substrate of LAPS chip, SiO<sub>2</sub> as the insulating layer and N<sub>3</sub>Si<sub>4</sub> as the H<sup>+</sup> sensitive material. The calibration solution used in the experiment selected 0.01mol/L sodium citrate as buffer, with 0.5mol/L NaCl as support electrolyte. The pH of solutions was adjusted between 4 and 9 with 0.1mol/L HCl and NaOH. Bias voltage range was -1000mV ~ +1000mV, scanning interval was 10mV with a scanning delay of 50ms. Detected I-V curves are shown in Fig.2a. As pH decreases, the I-V curve shifts to the left. Fig.2b shows that the bias voltage is highly correlated with pH ( $R^2=0.9868$ ) and the sensitivity is 53.69mV/pH, thus it fits in with cell acidification monitoring.

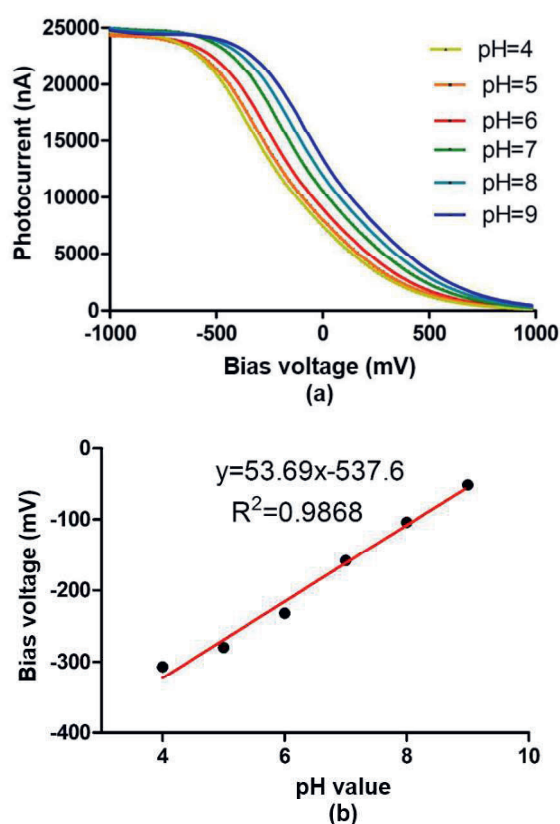


Fig.2. (a) IV curve of the pH gradient solution; (b) pH calibration curve.

### Cell Acidification Detection

Before the cell acidification experiments, the performance of LAPS was determined. Photocurrent amplification and repeatability were tested by the culture medium at pH 7.4, which is commonly used in the cell experiments. After verifying basic characteristics, the sensor unit was calibrated by the culture medium with pH 6, 7, 8, respectively, which is the working range in cell experiments. And each pH was recorded for hours to observe the stability of the sensor and calculated the mean of each pH data for calibration results. According to the

calibration curve, the actual photocurrent can be easily converted into corresponding pH value.

For seeding the cells directly on the LAPS, the chip was incubated with 5μg/ml laminin in phosphate buffer solution (PBS) for 1 h at 37 °C. Laminin improves the adhesion of cells onto the surface of the chip. Caco-2 cells in logarithmic growth phase were seeded onto LAPS chip with a density of  $4 \times 10^5$ /mL and incubated at 37°C, 5% CO<sub>2</sub> and saturated humidity for 24 h. After cells were attached to the chip, the medium used before was replaced by fresh medium with low buffer system. I-V curves were achieved every 5 minutes. The results (Fig.3) demonstrated the pH decrease in the process of cellular metabolism and the changing rates significantly decreased after 30 min, which means cell vitality is impacted.

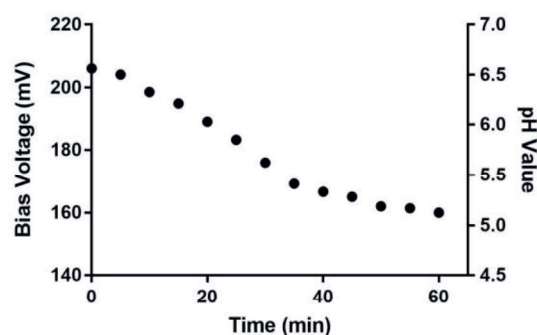


Fig.3. pH changes with time

### Conclusion

In this work, the performance of LAPS was determined by the calibration and cell experiments. The results presented that this system performed well in the real time detection of H<sup>+</sup>. Human colon adenocarcinoma cells Caco-2 were used for monitoring extracellular acidification rate. Therefore, it provides a new platform for cell vitality measurement.

### References

- [1] Castellarnau, M., et al. "Integrated cell positioning and cell-based ISFET biosensors." *Sensors and Actuators B: Chemical* 120.2 (2007): 615-620; doi: 10.1016/j.snb.2006.01.057
- [2] Werner, Carl Frederik, et al. "Determination of the extracellular acidification of Escherichia coli by a light-addressable potentiometric sensor." *physica status solidi (a)* 208.6 (2011): 1340-1344; doi: 10.1002/pssa.201001141
- [3] Hu, Ning, et al. "A novel microphysiometer based on high sensitivity LAPS and microfluidic system for cellular metabolism study and rapid drug screening." *Biosensors and Bioelectronics* 40.1 (2013): 167-173; doi: 10.1016/j.bios.2012.07.010