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A novel design of multifunctional integrated cell-based biosensors for simultaneously detecting cell acidification and extracellular potential

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ABSTRACT

The paper discussed a novel design of multifunctional cell-based biosensors for simultaneously detecting cell acidification and extracellular potential. Employing living cells such as cardiac myocytes as a source for the light addressable potentiometric sensor (LAPS) array, this cell-based biosensor was able to monitor both the acidification and extracellular potential in parallel. For LAPS array fabrication, part of the silicon base was heavily doped with boron to form separate testing areas. Detecting system was built involving lock-in amplifier and digital demodulation with FFT methods. This LAPS array showed a good sensitivity of 53.9 mV/pH to H^+ with good linearity. Each testing area for extracellular potential detection was decreased to 200 μm × 200 μm in size to obtain a better sensitivity. Experiment results showed that this LAPS array could monitor the acidification of cells as well as the extracellular potential with good sensitivity. This novel integrated biosensor will be useful for multi-parameter extracellular monitoring and can possibly be a platform for drug screening.

1. Introduction

In a living cell, thousands of biological reactions happen which are more or less coupled to each other and represent a complicated network. It is a different approach in functional online analysis of living cells in physiologically controlled environments for extended periods of time. A rigorous preselection of identified compounds by in vitro cellular screening is necessary prior to using the drug candidates for the further time consuming and expensive stage, e.g. in animal models. So nowadays, the aim is focused on integration of different microelectronic sensors into miniaturized biochips for a multifunctional online analysis of living cells.

Cell acidification and extracellular potential signals such as action potential are important parameters showing cell's status. Acidification plays an important role in cell metabolism. Usually, the extracellular pH change is used to indicate the living condition 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 a microchamber for cell experiments. Many attempts have been made to commercialize the LAPS system (Adami et al., 1995; Wolf et al., 1998). One successfully commercialized system using the LAPS for determination of the extracellular acidification of living cells is the Cytosensor Microphysiometer system, released in 1990 by Molecular Devices Corp. (Sunnyvale, CA).

To detect intracellular potentials, traditional glass pipettes with electrodes that put surround or in cells with the help of patch clamp will meet some problems such as high requirements in rigidity, diameters and impedance of electrodes. Besides, long-time detections cannot be performed since it is harmful to the cell (Hamill et al., 1981). Extracellular potential is usually measured employing field-effect transistor arrays (FET) (Sprössler et al., 1999) or substrate-integrated microelectrode arrays (MEA) (Connolly et al., 1990; Denyer et al., 1998). However, these two techniques both suffer from geometrical restriction and measurements are limited to a fix number of recording sites. LAPS is a surface potential detector with spatial resolution, based on silicon technology (Hafeman et al., 1988). With cells cultured on the surface of LAPS, extracellular potential can also be monitored (Xu et al., 2005). Comparing to MEA and FET, any desirable detection site can be freely selected by moving the light pointer across the surface of LAPS. Therefore, surface potential measurements are no longer restricted to predetermined sites.

The monitoring efficiency of extracellular parameters can be greatly improved by high throughput screening platforms. Due

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to the spatial resolving power, LAPS has an advantage for multi-sensing application (Shimizu et al., 1994). Usually, when used for multi-sensing, LAPS can detect several different ions' concentration in parallel (Men et al., 2005; Wang et al., 2005). However, when these LAPS array were used for cell analysis, only chemical signals but no potential signals were monitored. Chemical signals and potential signals of cells show great connections. Thus, it will be helpful for cell analysis if both kinds of information can be obtained.

In this work, we discussed a multifunctional cell-based biosensor, which can determine cell acidification and monitor the extracellular potential signals simultaneously. Fabrication of LAPS array as well as the detecting system was introduced. Cell experiments were presented to show the characteristics of the sensor and corresponding results were discussed in details.

2. Theory and sensor design

2.1. Light addressable potentiometric sensor (LAPS)

Light addressable potentiometric sensor with an electrolyte-insulation-semiconductor (EIS) structure is schematically shown in Fig. 1(a). Applying a dc bias voltage to the EIS structure, a depletion layer appears at the insulator (SiO_2)–semiconductor (Si) interface. Illuminating part of the surface with a modulated light induces a localized photo-induced current to be measured as a sensor signal, the amplitude of which depends on the local surface potential.

Characteristic *I–V* curve of n-type LAPS shows the relationship between the induced photocurrent and the bias voltage applied to the sensor chip (Fig. 1(b)). The regions of these sigmoid curves can be identified as follow. In the cut off region, there is no photocurrent. In the working region, the photocurrent rises almost linearly with decreasing voltage. The bias voltage is set to the point of the inflection of this curve and kept constant during the detection process. At this point the photocurrent is most sensitive to change in the surface potential. In the saturated region, the photocurrent is saturated, which means change of bias voltage within this range will not cause any change in photocurrent. Since the photocurrent changes with the bias voltage, if the bias voltage applied is kept constant, external potential changes coupled to the bias voltage can be determined by detecting the change of photocurrent.

For pH detection, a layer of Si_3N_4 is fabricated on the surface of LAPS. According to the site-binding theory (Siu and Cobbold, 1979; Bousse, 1982), a potential difference which is related to the concentration of H^+ in the electrolyte forms on the insulator (Si_3N_4/SiO_2)-solution interface. This potential is coupled to the bias voltage applied to the sensor. Larger concentration of H^+ provides a larger value of this potential difference, causing the *I–V* curve to shift along the negative half axis of bias voltage (Fig. 1(b)). When the bias voltage is kept constant in the middle region, change of the photocurrent indicates the pH change of the electrolyte.

LAPS is a surface potential detector with spatial resolution. Light pointer used for LAPS detection can be focused by microscope and optical lens, which suggests the LAPS possible for any desirable cell analysis. However, LAPS chip cannot be optimized to improve the spatial resolution substantially to the size of a single living cell (Parak et al., 1997), most likely to be between 30 and 100 μm, which means small cluster of cells or individual isolated cell can be chosen as the detecting object. After cells are cultured on the LAPS, a focused laser, 10 μm in diameter, is used to illuminate the front side of the chip to address the cells to be monitored. Excitable cells such as cardiac myocytes or neuron cells can generate extracellular action potential. This potential is coupled to the bias voltage applied to the LAPS, which correspondingly changes the amplitude of the photocurrent. Thus, by monitoring the photocurrent at a constant bias voltage, extracellular potential signals can be detected (Xu et al., 2005).

2.2. LAPS array

Illuminating different sensing areas with modulated lights of different frequencies generates a photocurrent signal, from which corresponding information of each testing site can be obtained by fast Fourier transform (FFT) methods (Cai et al., 2007). Comparing with conventional surface potential detectors such as FET or MEA, integration of LAPS array has many advantages. The most important feature of LAPS array is the great reduction of the required leads. For MEA, the number of required leads is the same as the number of electrodes, while for LAPS array, only one lead is necessary, regardless of the number of testing sites, which is important for high level integration (George et al., 2000). Besides, LAPS can detect extracellular potential as well as ion concentrations (Wu et

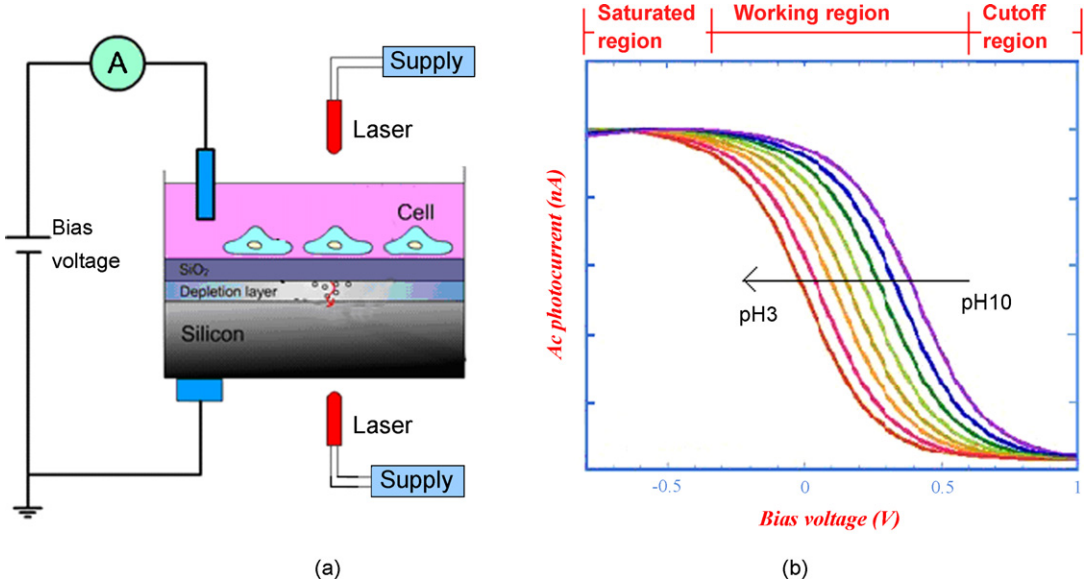

Fig. 1. Principle of the cell sensors based on LAPS. (a) The scheme of the LAPS. (b) Typical *I–V* curves of n-type LAPS.