Multiple Channel Detection of Cellular Activities by Ion Sensitive Transistors

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1. Introduction

Multiple channel detection of cellular activities by a light addressable potentiometric sensor (LAPS) or substrate-integrated microelectrode arrays (MEA) has been proposed to realize the high sensitive bio sensors such as Electric Nose [1-2]. However, these methods essentially have a lack of sensitivity because of using changes in photocurrent or gate capacitance. On the other hand, ion sensitive devices have the advantage that they utilize a region of drain current that varies drastically with fluctuation of the gate voltage, i.e. the subthreshold swing region. This is the well-known ion sensitive field effect transistor (ISFET) [3]. In spite of its favorable features, the multiple channel detection of cellular dynamics using high sensitive ISFET has not yet been demonstrated.

This paper reports on a multiple channel detection system for the human origin cellular activities using a specially designed bio-chip based on ISFET (Bio Transistor). The Bio Transistor we fabricated detects the reaction of cells stimulated by a reagent solution. Moreover, sensitive and simultaneous detection of cellular activities was achieved successfully with a resistive voltage-dividing circuit composed of several Bio Transistors connected in parallel.

2. Device Fabrication and Characteristics

Figure 1 schematically shows the operation principle of Bio Transistor. Cultured cells are arranged on the gate insulator instead of the gate electrode. When the cell become active through the stimulation of a reagent solution, ion channels open and then ions in the culture medium flow into the cell through the cell membrane. This shift of ion balance causes a shift in the ion current (I_j) and the junction potential (V_j) underneath the cells. Since the fluctuation of V_j causes variation of the gate signal in Bio Transistor, a difference in membrane potential is translated indirectly into an increase or decrease in the drain current.

Figure 2 (a) shows photograph of this fabricated Bio Transistor (chip size; 1.5 cm × 1.5 cm) with the cloning cylinder (Φ =9.5 mm). The channel length, gate oxide thickness and transistor width were L_{CH}=8 µm, T_{OX}=10 nm and W=50 µm, respectively. To ensure an adequate drain current, 400 transistors were connected in series. Human embryonic kidney cell transformed with adenovirus type 5 DNA, HEK293 were prepared for the recording of the cellular activities. Figure 2 (b) shows a photograph of cultured HEK293 cells of approximately 10 µm diameter, distributed uniformly on the manufactured Bio Transistor.

Figure 3 shows V_{GB} - I_D waveforms of Bio Transistor. A subthreshold swing region in V_{GB} - I_D curve appeared even

without the gate electrode. From this result, we estimated the optimum voltage conditions to be V_{GB} = -0.5 V to -1.0 V at V_{DS} =1.5 V.

3. Multiple Channel Detection System

In order to realize multiple channel observation, a new circuit system was established. Figure 4 (a) shows the measurement setup including four Bio Transistors connected in parallel. Bio Transistors were linked to the computer for system control via a signal processing circuit. Figure 4 (b) depicts the equivalent circuit of the measurement circuit. Each Bio Transistor was connected in series to resistance $R_D=1 \ k\Omega$. The output voltage V_{OUT} were passed through the voltage follower, the multiplexer, and the analog/digital converter within the signal processing circuit.

Figure 5 shows the time evolution of the fluorescence intensity of Ca^{2+} with the addition of 100 μL acethlcholine to HEK293. The increasing of fluorescence intensity which corresponds to the influx of Ca²⁺ into the cells was obtained with the addition of acetylcholine. Bio Transistors were observed under the following four conditions: using (1) with HEK293 cells and the addition of acetylcholine, (2) with HEK293 cells and the addition of isoprenaline, (3) with HEK293 cells and without the addition of a reagent solution, and (4) without HEK293 cells and with the addition of acetylcholine. In condition (2), isoprenaline was selected as a reagent solution alternative to acetylcholine because isoprenaline plays a major role in activation of an adenylate cyclase via G-proteins to generate c-AMP. Hence, we assumed that isoprenaline makes little contribution to the cellular response. In a similar way, potential fluctuation within the culture medium was expected not to occur in conditions (3) and (4) due to absence of the reagent solution and HEK293 cells, respectively. Thus, only under condition (1) was expected to fluctuate the membrane potential due to the metabolic reactions of cells by the acetylcholine. Figures 6 (a) to (d) show the time evolutions of I_D under each condition. As expected, I_D decreased rapidly with the addition of acetylcholine as clearly shown in Figure 6 (a). I_D decreased by approximately 70 μ A (8.0 % reduction).

4. Conclusions

We attempted the detection of cellular activities using a multiple channel measurement circuit containing several Bio Transistors. We confirmed that the drain current distinctly decreased only with the addition of acetylcholine to the cells. As a consequence, this multiple channel measurement system makes it possible to detect the cellular activities sensitively and simultaneously.



Fig. 1 Operation principle of Bio Transistor.



Fig. 2 Photograph of fabricated Bio Transistor; (a) cloning cylinder attached to chip and (b) cultured HEK293 cells.







Fig. 5 Time evolution of fluorescence intensity of HEK293 cells in calcium ion assay.

References

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Fig. 4 Measurement setup of multiple channels detection system composed of four Bio Transistors.



Fig. 6 Time evolutions of drain current under different conditions of reagent and HEK293 cells. Arrow indicates time of addition of reagent solution.