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Electrical detection of drug transport at cell membrane using oocyte-based field effect devices

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Abstract We propose the non-label and invasive analysis method for drug transport using an oocyte-based field effect device, which is based on potentiometric detection of extracellular potential change derived from exchange of molecular charges at cell membrane/gate insulator. The extracellular potential change at cell membrane/gate insulator can be directly transduced into electrical signal such as threshold voltage changes using the oocyte-based FET. The time course of threshold voltage change can be monitored during the uptake of common substrate through the transporter at the cell membrane without any labeling materials. The platform based on the oocyte-based FET is suitable for a simple and convenient system for drug screening in pharmaceutical lead discovery.

Key words: field effect transistor, oocyte, transporter, drug screening

1. INTRODUCTION

We have been investigating a new device for detection of bio-molecular recognition events using field effect devices, which are based on potentiometric measurement of molecular charges at a gate insulator/solution interface. So far we have succeeded in a direct detection of various DNA recognition events such as hybridization, intercalation, primer extension and so on, and have found further the possibility of single nucleotide polymorphism (SNP) typing and DNA sequencing using field effect devices [1-7]. In this paper, we propose a cell-based field effect devices such as an oocyte-based field effect transistor (oocyte-based FET) for drug transport analysis, which is a FET combined with *Xenopus laevis* oocyte (**Figure 1**), and investigate the electrical characteristics of oocyte-based FET with a transporter expressed at the oocyte membrane. In addition, we report on the possibility of non-invasive cell analysis and drug screening using the oocyte-based FET.

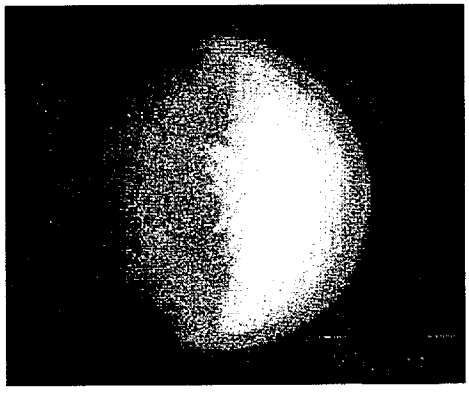

Figure 1. *Xenopus laevis* oocyte

2. METHODS

Insulated gate field effect transistors are fabricated using the standard integrated circuit technology except for deposition of the gate electrode. Four n-channel depletion mode FETs and a temperature sensor are integrated in a 5 mm x 5 mm chip (**Figure 2**). The thicknesses of the Si_3N_4

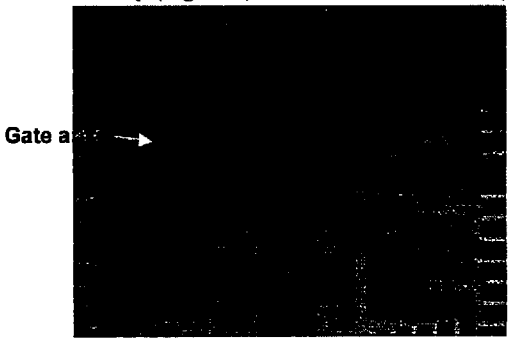

Figure 2. Fabricated field effect transistor.

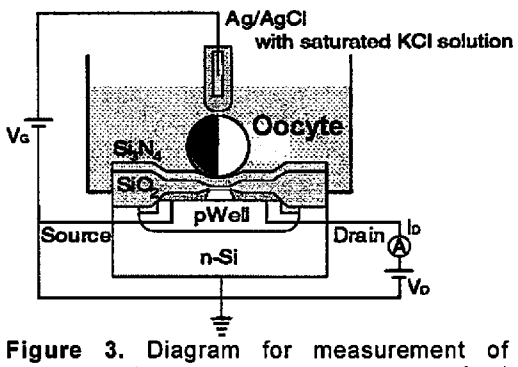

Figure 3. Diagram for measurement of electrical characteristics using oocyte field effect transistor.

layer and the SiO_2 layers are 140 nm and 35 nm, respectively. The fabricated FET chip was mounted on a flexible polyimide film with patterned copper electrodes and wire-bonded. The FET chip was encapsulated with an epoxy resin (ZC-203, Nippon Pelnox) except for the gate areas. Both the fabricated FETs and the commercial ISFETs (BAS Inc.) were used for the experiments in the present study. The FETs were immersed in a phosphate buffer solution (0.025 M Na_2HPO_4 and 0.025 M KH_2PO_4, pH 6.86, Wako) with an Ag/AgCl reference electrode with saturated KCl solution and oocyte was placed on the gate area of FETs (**Figures 2 and 3**). The electrical characteristics of the FETs such as the gate voltage (V_G)-drain current (I_D) characteristics were measured in a pH 6.86 phosphate buffer solution at room temperature using a semiconductor parameter analyzer (4155C, Agilent). The threshold voltage shift ΔV_T was determined after introduction of common substrates to oocyte-based FETs. The ΔV_T was defined as a difference of the V_G-I_D characteristics at a constant drain current of 700 μA. The time course of ΔV_T during uptake of common substrates was monitored using a circuit with which the potential change at the interface between an aqueous solution and the gate insulator can be read out directly at a constant drain current. In the present study, the gate voltage and the drain current were set to be 1 V and 700 μA, respectively.

We have prepared two types of oocyte-based FETs; one is the transportocyte FET with human organic anion transporting peptide 2 (hOATP2) at the cell membrane; another is the control oocyte FET without it. Using these oocyte-based FETs, differential measurements were performed in order to eliminate the common background noises such as temperature change, change in ion concentration and so on (**Figure 4**). Also, the reference FET without oocyte was prepared in order to take account of the effect of oocyte placed at the gate surface of the oocyte FET.

3. RESULTS AND DISCUSSION

When an estrone-3-sulfate (E3S) as a common substrate was introduced into two kinds of oocyte-based FETs and the reference FET, the interfacial potential of the transportocyte FET increased with uptake of E3S mediated by hOATP2 while the control oocyte FET and the reference FET showed little interfacial potential change, corresponding to the V_T shift (**Figure 5**). This result was in

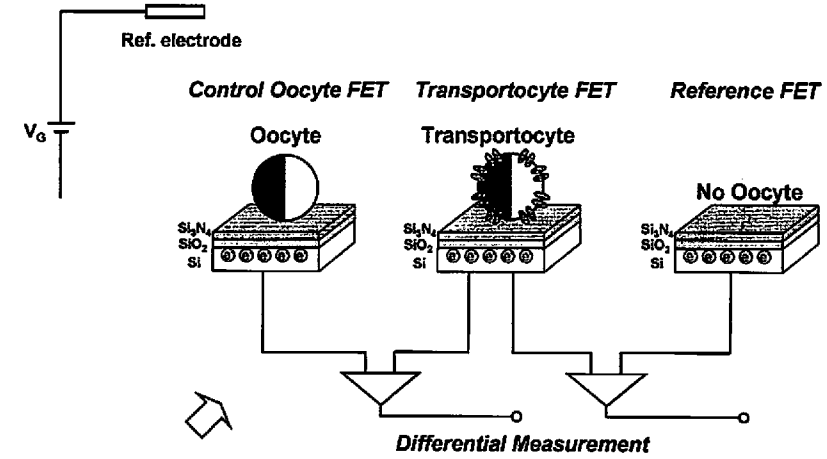

Figure 4. Differential measurements of oocyte-based FETs. The back ground noises such as temperature change and so on can be eliminated.

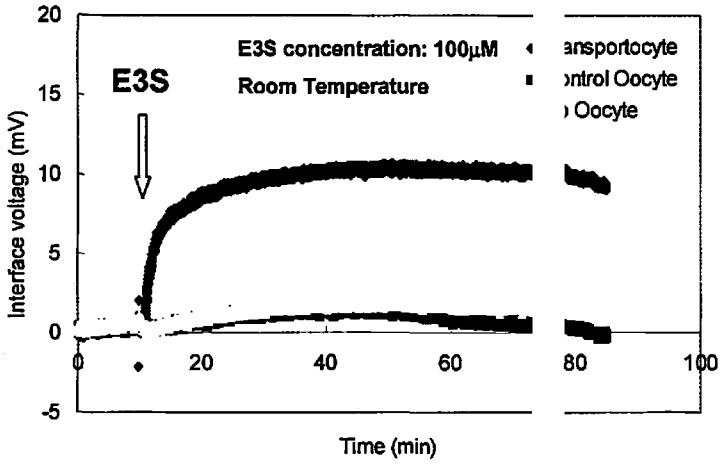

Figure 5. Direct detection of uptake of E3S substrate using the oocyte-based field effect transistors.

agreement with the experimental results performed by BD Gentest, Inc.[8], which were based on uptake of [^{3}H]-labelled E3S mediated by hOATP2. Moreover, we could realize the quantitative analysis of E3S uptake using oocyte-based field effect devices (**Figure 6**), which was estimated as the flat band voltage V_F shifts in capacitance(C)-voltage(V) characteristics using the field effect device different from transistor. The small change of control device without oocyte on the gate was based on the background noises caused by temperature change, change in ion concentration and so on. Therefore, we have found out the possibility of a label-free and non-invasive drug transport screening using the oocyte-based field effect devices such as the oocyte FET, although what kinds of ions or charged molecules contribute to the interfacial voltage change of the oocyte-based FET with transporter is under investigation.

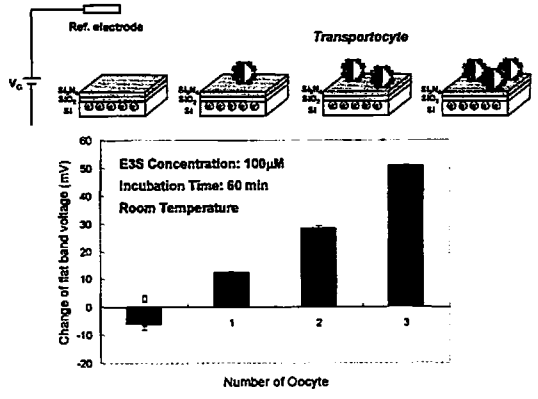

Figure 6. Quantitative analysis of uptake of E3S substrate using oocyte field effect devices.

4. CONCLUSIONS

We have shown the label-free and non-invasive analysis method for drug transport screening using the oocyte-based FET, which is based on potentiometric detection of extracellular potential change derived from exchange of molecular charges mediated by transporting peptides at cell membrane. The interfacial voltage change could be monitored during the uptake of common substrate mediated by the transporter without labeling materials and fracturing oocytes. The platform based on the oocyte-based FET is suitable for a safe, fast and convenient detection system for drug screening in pharmaceutical lead discovery.

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