Organic TFT-based Biosensors Functionalized with Artificial Receptors

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ABSTRACT

We have studied organic thin-film transistors (OTFTs) functionalized with artificial receptors as a new sensing platform for a variety of targets such as small ions and molecules, and biomacromolecules. Herein, the detection of biogenic amines by OTFT and real-time monitoring of glucose by OTFT integrated microfluidic system are demonstrated.

1. INTRODUCTION

Detection of biologically abundant compounds is of great importance in clinical diagnostic investigations. Immunoassays have been applied for facilitating and easing the sensing systems.[1] Furthermore, the assays combined with many types of apparatuses such as surface plasmon resonance (SPR)[2], quartz crystal microbalance (QCM)[3] and metal-oxide-semiconductor field-effect transistor (MOSFET)[4] enable sensitive and selective detection of the compounds based on the specific antigen-antibody interactions. Although the immunoassays are applicable for variety of compounds (e.g. small molecules and proteins), complicated and time-consuming pre-treatment process limit their application.

In this regard, we decided to employ combination of OTFTs with functionalized artificial receptors as a new platform for detection of biologically abundant compounds. Molecular recognition of this system relies on the obtained electrical signals caused by the interaction between the artificial receptors and the target analytes, and allows sensitive and selective detection by OTFTs. We have previously reported the highly sensitive OTFTs for detection of proteins[5] including serum albumin[6] and phosphorylated protein.[7] In this work, to expand the scope of the application of OTFTs, we report the detection of biologically relevant small molecules such as histamine by an electrolyte-gated OTFT-based biosensor. Histamine has significant role in neurotransmission[8] and scombroid food poisoning.[9] Thus, sensitively sensing of it is in high demand for medicinal and food chemistry. In addition, an extended gate-type OTFT with microfluidic system to realize the real-time monitoring of biomolecules is investigated. As a first step for the real-time monitoring, we selected glucose (Glc) due to its vital role in health and diseases.

2. EXPERIMENTAL

Detection of biogenic amines by the electrolyte-gated *OTFT*

Electrolyte-gated OTFT was fabricated to detect biogenic amines in aqueous solution (Fig. 1).[10] Gold (Au) was used in fabrication of source, drain, and gate electrodes. A carboxy side-chain attached polythiophene, (poly{3-(5-carboxypentyl)thiophene-2,5-diyl}, P3CPT) was used as semiconductor and molecular recognition moiety. Detection of biogenic amines was performed in 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer containing 100 mM NaCl. To compare the changes of the device characteristics upon the addition of the biogenic amines (Fig.1(a)), the obtained current was normalized as $(I–I_0) / I_0$. I and I_0 are representing the observed drain current of the OTFT upon the addition of

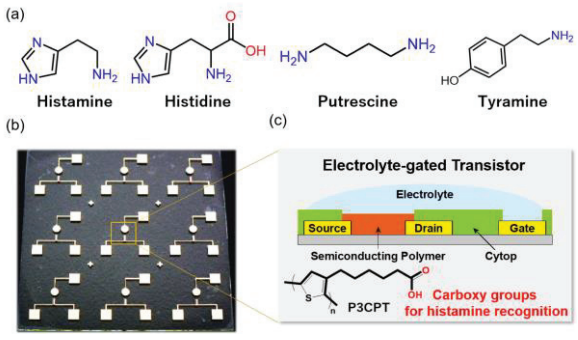

Fig. 1 (a) Structures of main analytes. (b) A photograph showing the fabricated OTFTs (nine devices in the picture). The scale bar corresponds to 1 cm. (c) Schematic illustration of the OTFT with an electrolyte (an analyte solution).

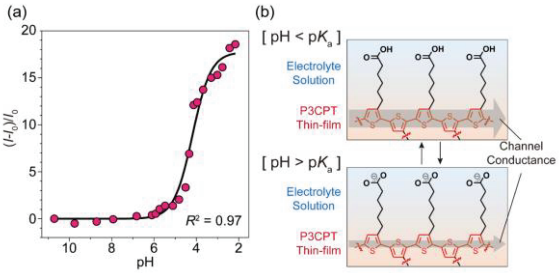

Fig. 2 (a) pH-dependency of the relative drain current of the electrolyte-gated OTFT with water containing NaCl (100 mM) at r.t. Applied voltages at the gate and the drain were 0.3 V. (b) A plausible mechanism of the current response in the OTFT induced by pH changes. Deprotonation at the carboxy-terminated side-chain of P3CPT induces a decrease of the conductance within the OTFT.

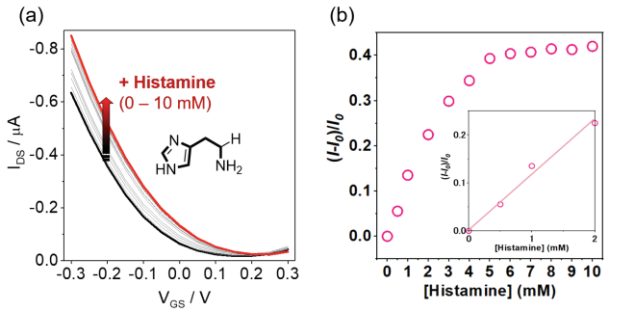

Fig. 3 Transfer characteristics (I_{DS}–V_{GS}) of the electrolyte-gated OTFT upon addition of increased amounts of histamine in a MES buffer solution (100 mM) with NaCl (100 mM) at pH 5.5. at r.t. [Histamine] = 0–10 mM. (b) Titration isotherm corresponding to the histamine-induced current change. The inset shows the lower end of the titration. The relative standard deviation (RSD) of each response is ≦7%.

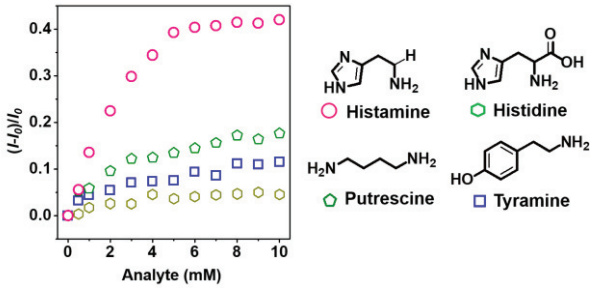

Fig. 4 Changes in the drain current of the electrolyte-gated OTFT due to analytes at various concentrations in a MES buffer solution (100 mM) with NaCl (100 mM) at pH 5.5. Histamine (pink circles), putrescine (green pentagon), tyramine (blue square), and histidine (olive hexagon). The RSD of each response is 8%.

an analyte and the drain current in the absence of an analyte, respectively.

Real-time Glc monitoring by extended gate-type OTFT combined with microfluidic system

In this case, Au-made source and drain electrodes, and an aluminum (Al)-fabricated gate were utilized. A π-conjugated polymer derivative, poly{2,5-bis(3-hexadecylthiophene-2-yl)thieno[3,2-b]}thiophene (PBTTT-C16) was casted as a semiconductor layer. The fabricated extended gate-type OTFT was connected with microfluidic system for real-time monitoring of Glc concentration. For the first step, 20 mM Glc solutions in 100 mM PBS (pH 7.4) containing 100 mM NaCl were injected and time course of the electrical signals were continuously monitored. To realize the real-time monitoring of the Glc concentration in biological system, a pesudo model for Glc release and consumption was established. Time courses of electrical signal upon increase and decrease in amount of Glc were tracked within a concentration range from 0 to 20 mM.

3. RESULTS AND DISCUSSION

Detection of biogenic amines by the electrolyte-gated *OTFT*

To evaluate the chemical stimuli-responsivity of the OTFT, we examined the pH dependency of the drain current because protonation/ deprotonation of the carboxy moieties of the P3CPT should affect the electrical properties of the fabricated OTFT. As expected, the drain current of the fabricated OTFT depended on the pH of the electrolyte solution (Fig. 2(a)). Based on results of the pH titration, the pKa value of P3CPT was determined to be 4.2. The observed pH dependency could be explained by the double-layer model indicating that the channel conductance of the TFT could be decreased by ionization of the carboxy moieties of P3CPT at the semiconductor/electrolyte interface (Fig. 2(b)).

The successful chemical-stimuli responsiveness in the OTFT encouraged us to perform the electrical detection of biogenic amines. In this regard, histamine was recognized at binding sites consisting of three amino acid residues (Asp186, Asp98, and Thr190) a H2 histamine receptor (H2-HR) meaning that the H2-HR can recognize histamine through non-covalent interactions (hydrogen bonding and electrostatic interactions) with carboxylates.[11]

As such, multi-noncovalent interactions that stemmed from carboxylates could be employed for sensing biogenic amines. Upon addition of histamine in a MES buffer solution, we observed distinct amplification in the drain current correlated with histamine concentration (Fig. 3(a)). The observed increase probably would be a neutralization between the negatively charged carboxy group (Fig. 3(a)) of P3CPT and positively charged ammonium group of histamine. The limit of detection (LOD) was estimated to be LOD = 1.6 mM. Since the estimated sensitivity of the electrolyte-gated OTFT for histamine is within the practical concentration range for scombroid food poisoning,[9] the OTFT device is capabale for food safety assessments.

Finally, the selectivity of the electrolyte-gated OTFT was investigated using a mixture containing histamine, putrescine, tyramine and histidine (Fig. 4). The electrolyte-gated OTFT device responded to analytes in the following order: histamine >> putrescine > tyramine > histidine. The strongest response of the fabricated device was to histamine among the analytes, while putrescine, tyramine and histidine showed weaker responses. Control experiments with monoamine derivatives (= methylamine, dimethylamine, and trimethylamine) and amino acids suggested that the observed selectivity of the P3CPT-based OTFT sensor is derived from multi-interactions at the semiconductor/electrolyte interface. We believe that these data validate the potential of electrolyte-gated OTFTs as a new promising platform for chemical sensing applications.

Real-time Glc monitoring by extended gate-type OTFT combined with microfluidic system

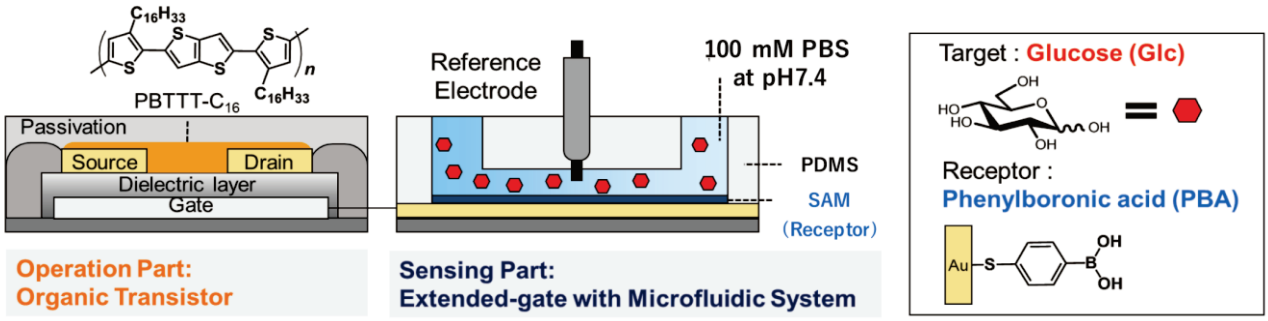

Fig. 5 Schematic illustration of an extended-gate type OTFT connected with a microfluidic system for real-time glucose monitoring.

To operate in low-voltage and prevent the electrical degradation of the OTFT during the long-term monitoring of Glc concentrations, we designed the device composed of two parts; the drive part (i.e. OTFT) and the detection part (i.e. extended-gate electrode) (Fig. 5). In order to establish continuous and quantitative sensing systems, the detection part in OTFT is coupled with microfluidic system.

In this system, we first investigated the ability of the device to detect continuous changes of Glc concentrations in a real-time approach. To allow real-time monitoring, we applied constant voltages for gate and drain electrodes, respectively at −1.5 and −1 V. Fig. 6 shows the detection of electrical signals after each injection of Glc solution. The drain current (I_{DS}) was plateau before addition of the Glc solution, whereas dramatical increase of the current was observed after addition of the Glc solution. The current increase arises from the negative charges formed by boronate esterification between PBA and Glc, affecting the transistor properties. This is supported by the result that drain current decreased by washing the electrode with PBS (removal of bound Glc).

For real applications, we attempted to monitor the pseudo Glc consumption and release of liver cells using the microfluidic OTFT sensor by modifying the Glc concentration in solution during the experiment. Fig. 7 shows a relationship between the time-dependent I_D and gradual concentration changes of Glc mimicking a pseudo Glc consumption of liver cells. The I_D was gradually decreased according to Glc concentrations, meaning that the real-time monitoring of Glc concentrations was successfully achieved. Fig. 8 shows a progressive increase of the I_D owing to a rise of the Glc concentration. Thus, the developed system is also able to detect the evolution of Glc concentration reproducing a pseudo Glc release of liver cells. In addition, we also successfully monitored random changes (i.e. mimicry of the actual cell activities) of the Glc concentration.[12] According to a previous report about the Glc metabolism in liver cells such as HepG2 ones, the response time (80% response time: ≈ 2 min) of the fabricated sensor system is certainly enough to apply for monitoring of cell activities.[13] These observations demonstrate that our microfluidic systems

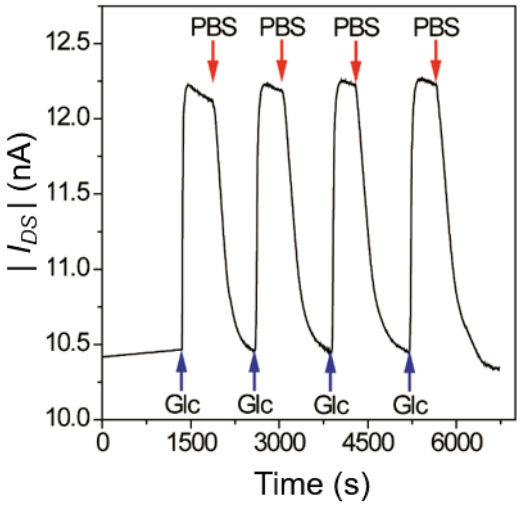

Fig. 6 Time course changes of the I_{DS} with (a) an increase or a decrease in the concentration of Glc (20 mM) in the PBS buffer solution (100 mM) with NaCl (100 mM) at pH 7.4. V_{DS} = −1 V, V_{GS} = −1.5 V. Flow rate of the injection was 25 μL/min.

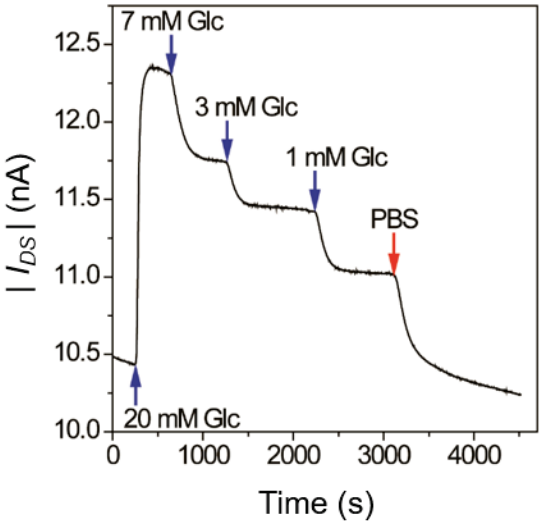

Fig. 7 A pseudo Glc consumption of liver cells by decreasing the concentration of Glc during the real-time monitoring in the PBS buffer solution (100 mM) with NaCl (100 mM) at pH 7.4. V_{DS} = −1 V, V_{GS} = −1.5 V. Flow rate of the injection was 25 μL/min.

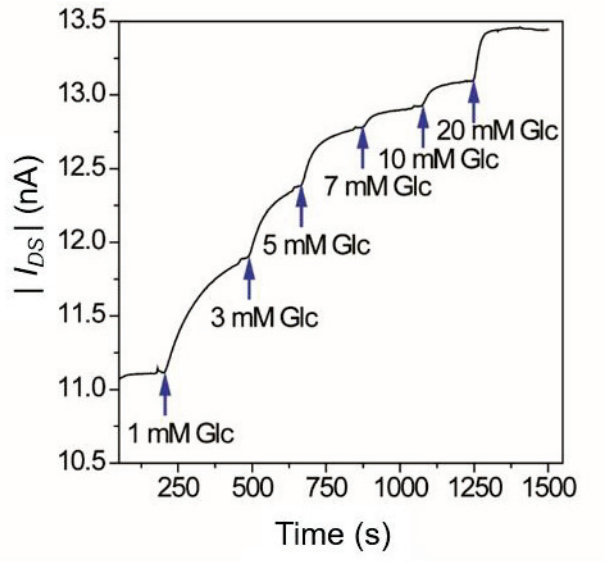

Fig. 8 a pseudo Glc release of liver cells in the PBS buffer solution (100 mM) with NaCl (100 mM) at pH 7.4. V_{DS} = −1 V, V_{GS} = −1.5 V. Flow rate of the injection was 25 μL/min.

have the potential for monitoring of release and uptake of Glc by cells.[14] We hope that this open up a new vista for detection of Glc and preventing Glc related diseases.

4. CONCLUSIONS

In this study, we introduced two systems including electrolyte-gate OTFT and extended gate-type OTFT coupled with microfluidic device for detection of biogenic amines and glucose, respectively. Biogenic amines were successfully detected by the electrolyte-gated OTFT with ultra-low voltage below |0.3| V. Interestingly, high selectivity for histamine over other kinds of the amines were observed. In the model of Glc release and consumption, current changes due to increased and decreased concentration of Glc were observed. The results obtained indicate that the fabricated device would be used in the monitoring of the bioactivity liver cells. In our belief, the OTFTs functionalized with artificial receptors are capable to contribute to the progress of OTFT-based biosensors.

5. REFERENCES

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