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Human influenza virus detection using sialyllactose-functionalized organic electrochemical transistors

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ABSTRACT

An organic electrochemical transistor (OECT) with a trisaccharide-grafted conductive polymer channel was developed for human influenza A virus detection under aqueous conditions. A target recognition element was introduced into the electrochemical amplifier of the OECT for highly sensitive, selective, and label-free virus sensing. 3,4-Ethylenedioxythiophene (EDOT) and its derivative bearing an oxylamine moiety (EDOTOA) were electrochemically copolymerized on the channel region composed of a PEDOT:PSS thin film. The trisaccharides composed of Sia-α2,6′-Gal-Glu (2,6-sialyllactose), a specific receptor for the hemagglutinin used as a spike protein on the surface of human influenza A virus, were covalently introduced into the EDOTOA unit. Changes in the drain current of the OECT were observed following virus adsorption onto the 2,6-sialyllactose-functionalized channel. A signal transduction mechanism involving a doping effect due to the adsorption of negatively-charged virus nanoparticles is proposed. The limit of detection was more than two orders of magnitude lower than commercial immunochromatographic influenza virus assays over the same detection time. Because of its processability with printing technologies and low power consumption, the OECT device developed here may be suitable for the wearable monitoring of influenza virus infection.

1. Introduction

Organic electrochemical transistors (OECTs) have attracted much attention in recent years because they can be stably operated in contact with an electrolyte at relatively low operating voltages for chemical and biological sensing [1–5]. The operation mechanism of OECTs is based on electrochemical doping/dedoping processes because ions in the electrolyte can move into and out of the channel made of conducting polymers depending on the gate bias due to the absence of insulating layers. When positive bias is applied to the gate, cations migrate from the electrolyte to the channel. As a result, the hole density decreases in the channel and the drain–source current decreases [6]. The ion/electron conversion characteristic of OECTs is useful for recording electrical and ionic signals in biology. In addition, conductive organic materials have advantages such as low cost, flexibility, processability, and low cytotoxicity [7–12]. OECTs can be fabricated on various substrates including flexible sheets, rubber, and textiles [5,13–16]. Because electrochemical doping/dedoping occurs in the whole matrix of the conducting polymer channel, the device usually exhibits high sensitivity [4]. To date, OECTs have been used to sense cell activity [17–19], protein [20,21], DNA [22,23], dopamine [24], glucose [25–28], ions [29], and various biologically relevant analytes [30] in a label-free manner.

Poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS) is widely used for constructing the channel part of OECTs [31]. There are two main approaches for endowing biorecognition ability on this material: the introduction of bioactive dopant such as heparin, and the chemical derivatization of PEDOT [31]. Physical doping of bioactive molecules is easy, but suffers from the control of ligand density in the matrix due to the inevitable release of the dopant into the bulk solution over time [12]. While the covalent introduction of a bio-recognition site onto the side chain of PEDOT can control and maintain the ligand density as well as the electrical conductivity through existing chemical methods [31]. To date, a wide range of synthetic and biological ligands including enzymes [32,33], antibodies [34,35], nucleic acids [36,37], and sugars [38] have been successfully conjugated to conducting polymers for specific applications.

In this study, detection of whole human influenza A virus was performed using an OECT. According to World Health Organiza-

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tion (WHO) estimates, influenza virus infects 5%–15% of the world's population, bringing about 250–500 thousand deaths every year [39]. Efficient detection methods are therefore vital. The identification of influenza virus subtypes by genome analysis is important for preventing the outspread of highly pathogenic influenza virus infections in humans. A conventional diagnostic tool for seasonal flu at clinics is an immunochromatographic test [40]. Because anti-influenza virus therapeutic drugs are effective within 48 h after onset, early diagnosis is crucial for medication [41]. Therefore, improvements in sensitivity are required for point-of-care testing. Hemagglutinin protein is a glycoprotein that forms a trimer on the surface of the influenza virus envelope. Binding of hemagglutinin to the host cell surface during viral invasion is mediated by sialic acid-terminated trisaccharide receptors on the cell membrane [42,43]. The viral hemagglutinin as an antigenic epitope specifically recognizes the linkage between sialic acid and lactose residues in the trisaccharide. Human influenza A virus (H1N1) preferentially binds to sialyllactose via an α2,6′ linkage (2,6-sialyllactose) and avian influenza virus binds to sialyllactose via an α2,3′ linkage (2,3-sialyllactose) [42]. Based on this biochemistry, here we aimed to develop 2,6-sialyllactose-functionalized OECT biosensors for the specific detection of human influenza A virus. Previously, we developed a conducting copolymer composed of 3,4-ethylenedioxythiophene (EDOT) and its adduct bearing an oxylamine group (EDOTOA) for potentiometric sensing of the virus [38]. Oxylamine spontaneously reacts with the reducing end of 2,6-sialyllactose during the bioconjugation reaction. Prompted by the development of wearable sensors for human influenza A virus, we now aim to acquire a reliable amperometric signal following viral recognition using a trisaccharide-functionalized conducting polymer in the OECT setup. To introduce 2,6-sialyllactose into the channel region of the OECT, EDOTOA and EDOT were electrochemically copolymerized onto the spin-coated PEDOT:PSS film. This process generates a composite of poly(EDOTOA-co-EDOT)/PEDOT:PSS. The specific recognition of human influenza A virus was monitored by changes in the drain current of the OECT.

2. Materials and methods

2.1. Reagents

An interdigitated array electrode on glass substrate was purchased from BAS (Tokyo, Japan). PEDOT:PSS (1.3 wt% dispersion in H_2O) was purchased from Sigma-Aldrich Japan (Tokyo, Japan). (3-glycidyloxypropyl)-trimethoxysilane, 2,3-sialyllactose sodium salt, and 2,6-sialyllatose sodium salt were purchased from Tokyo Chemical Industry (Tokyo, Japan). Sodium perchlorate ($NaClO_4$) and Dulbecco's phosphate buffered saline (DPBS) were purchased from Wako Pure Chemicals (Tokyo, Japan). Human influenza A virus (H1N1, A/PR/8/34) was inoculated into 11-day-old chicken embryos at 37 °C. The allantoic fluid was collected 3 days after inoculation and was filtered through 0.45 μm syringe filter. The influenza virus was inactivated by fixation in 0.05% formalin and was used without further purification. All other reagents of analytical grade were purchased from commercial sources and were used without further purifications. Milli-Q water (Merck Japan, Tokyo, Japan) was used throughout the study.

2.2. Preparation of the OECT device

The array electrode was cleaned with an O_2 (15 mL min^{-1}) and Ar (15 mL min^{-1}) mixture of gases plasma (PDC210, Yamato Scientific. Tokyo, Japan) at 300 W for 60 s. Next, areas, except for the interdigitated array, were masked with silicone tape. Then, the PEDOT:PSS solution containing 1 wt% (3-glycidyloxypropyl)-trimethoxysilane was spin-coated (MSA100, MISAKA, Tokyo, Japan) onto the electrode at 1000 rpm, followed by annealing at 140 °C for 1 h. (3-Glycidyloxypropyl)-trimethoxysilane is a surface adhesion promoter and polymer cross-linker for enhancing the stability of the PEDOT:PSS film in aqueous environments [44]. EDOTOA is an EDOT derivative bearing an oxylamine moiety for covalent linkage with sialyllactose via the glycosylation reaction. The synthesis procedures for EDOTOA were described in our previous work [38].

Electropolymerization was performed in an aqueous mixture of EDOTOA and EDOT (10 mmol L^{-1}) at a desired composition containing 100 mmol L^{-1} $NaClO_4$ at room temperature. The spin-coated PEDOT:PSS film as a working electrode, an Ag/AgCl (in 3.3 mol L^{-1} KCl with a salt bridge) reference electrode, and a platinum wire counter electrode were dipped in the solution. Then, two consecutive cycles of cyclic voltammetry were performed from −0.6 to +1.1 to −0.6 V at the scan rate of 0.1 V s^{-1} using a Versa STAT 3 potentiostat (Princeton Applied Research, Oak Ridge, TN, USA). Thereafter, the poly(EDOTOA-co-EDOT)/PEDOT:PSS film was soaked in a solution of 2,6-sialyllacotse (Sia-α2,6′-Lac) or 2,3-sialyllacotse (Sia-α2,3′-Lac) (100 μmol L^{-1} in acetic acid, pH 5.3) for the glycosylation reaction at 60 °C for 12 h.

2.3. Characterization

Drain–source current versus drain–source voltage (I_d vs. V_d) and drain–source current versus gate voltage (I_d vs. V_g) characteristics were measured using a semiconductor parameter analyzer (4155C, Agilent, Tokyo, Japan). All measurements were conducted in a hundred times diluted (0.01×) DPBS at pH 7.4 and 25 °C using an Ag/AgCl (in 3.3 mol L^{-1} KCl with a salt bridge) gate electrode.

The surface morphology of the conducting polymer films was observed after carbon coating by scanning electron microscopy (SEM, S-3400N, Hitachi, Tokyo, Japan) with an acceleration voltage of 5 kV, a working distance of 9800 μm, and an emission current of 86 μA.

Elemental analysis on the surface of the film was performed by X-ray photoelectron spectroscopy (XPS, AXIS-HSi165, Shimadzu–Kratos, Kyoto, Japan) with a 15 kV Mg–Kα light source, and the take-off angle of the photoelectrons was set at 90°. Mean and standard deviation (SD) values were obtained from measurements at three positions.

The amount of 2,6-sialyllactose introduced onto the poly(EDOTOA-co-EDOT)/PEDOT:PSS was measured using a NAPiCOS quartz crystal microbalance (QCM) twin sensor (PSA-SB-3002T, Nihon Dempa Kogyo, Tokyo, Japan) at 25.00 ± 0.02 °C. The fundamental frequency was 30 MHz. Mean and SD values were obtained from three measurements.

2.4. Human influenza virus sensing

The 2,6-sialyllactose- or 2,3-sialyllactose-grafted poly(EDOTOA-co-EDOT)/PEDOT:PSS channel and the Ag/AgCl (in 3.3 mol L^{-1} KCl with a salt bridge) gate electrode were immersed in 0.01 × DPBS (pH 7.4) buffer for 30 min at room temperature. Then, a stock solution of human influenza A virus was sequentially added every 10 min to the solution to give final concentrations of 0.015, 0.03, 0.06, 0.12, 0.25, 0.5, and 1 hemagglutination units (HAU). One HAU is defined as the minimum amount of virus that will cause complete agglutination of the erythrocytes. The OECT with the poly(EDOT)/PEDOT:PSS channel served as a control. A low-noise DC power supply (Xitron 2000, Toyo Corporation, Tokyo, Japan) was used for applying the drain–source and source–gate voltages. A digital multimeter (34401 A, Agilent, Tokyo, Japan) and bundled BenchVue software (BV0001 B-1 TP, Keysight, Tokyo, Japan) were used for monitoring the time course of the drain current. The drain