Chapter 14

Highly Sensitive Glucose Sensor Based on Organic Electrochemical Transistor with Modified Gate Electrode

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Abstract

An organic electrochemical transistor (OECT) with a glucose oxidase (GOx) and poly(n-vinyl-2-pyrrolidone)-capped platinum nanoparticles (Pt NPs) gate electrode was successfully integrated with a microfluidic channel to act as a highly sensitive chip-based glucose sensor. The sensing mechanism relies on the enzymatic reaction between glucose and GOx followed by electrochemical oxidation of hydrogen peroxide (H_2O_2) produced in the enzymatic reaction. This process largely increases the electrolyte potential that applies on PEDOT:PSS channel and causes more cations penetrate into PEDOT:PSS film to reduce it to semi-conducting state resulting in lower electric current between the source and the drain. The extremely high sensitivity and low detection limit (0.1 μM) of the sensor was achievable due to highly efficient Pt NPs catalysis in oxidation of H_2O_2. Pt NPs were deposited by a bias-free two-step dip coating method followed by a UV-Ozone post-treatment to enhance catalytic ability. A polydimethylsiloxane (PDMS) microfluidic channel was directly attached to the OECT active layer, providing a short detection time (~1 min) and extremely low analyte consumption (30 μL). Our sensor has great potential for real-time, noninvasive, and portable glucose sensing applications due to its compact size and high sensitivity.

Key words Organic electrochemical transistor, Biosensor, Glucose detection, Microfluidics, Platinum nanoparticles

1 Introduction

Organic thin film transistors (OTFTs) are increasingly used as biosensor platform linking organic electronics and biological applications [1, 2]. Organic electrochemical transistors (OECTs) are an important type of OTFTs that have attracted much interests as biological sensors due to their stability in an aqueous environment, low operation voltage and high sensitivity to biological and chemical analytes. OECTs, first introduced by Wrighton et al. [3], are three terminal devices with drain, source, and gate electrodes. PEDOT:PSS or poly(3,4-ethylenedioxythiophene) polystyrene sulfonate is a transparent, conductive polymer that is a mixture of two ionomers. Together the charged macromolecules form a

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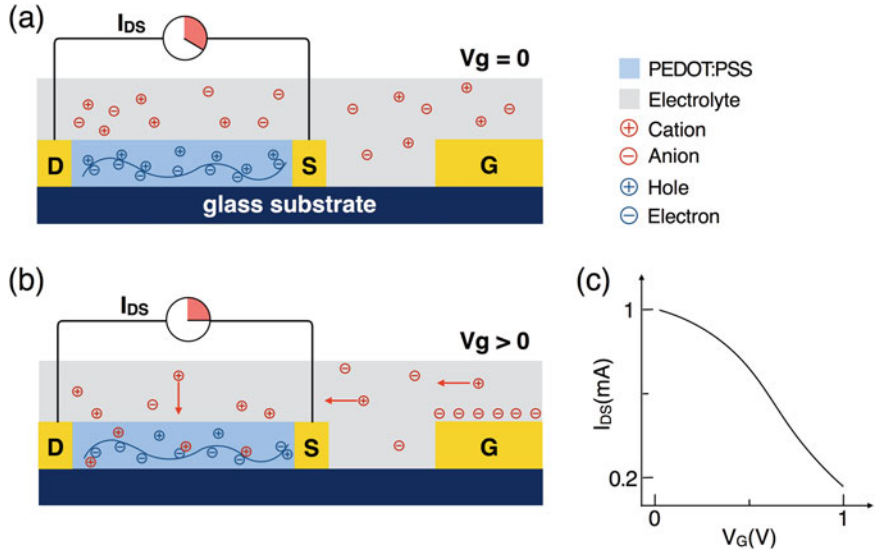

Fig. 1 (**a**) Ion distribution in OECT without gate bias. (**b**) Ion distribution in OECT under positive gate bias. (**c**) Typical transfer characterization of OECT device

macromolecular salt. In OECTs, PEDOT:PSS serves as an active channel linking the drain and source electrodes together. The third gate electrode is separated from the channel by an electrolyte. The working mechanism of OECTs relies on the de-doping process of the active channel induced by the positive gate bias. The PEDOT:PSS film, in its pristine state, is a mixture of doped (oxidized) and undoped (neutral) PEDOT units, when there is no gate bias (Fig. 1a), PEDOT:PSS is in its conducting form ($PEDOT^+$). After the addition of gate bias (Fig. 1b), cations penetrate into PEDOT:PSS film due to the electrical field. $PEDOT^+$ is reduced to the semi-conducting state ($PEDOT^0$) according to the reaction below:

$$PEDOT^+PSS^- + M^+ + e^- \rightleftharpoons PEDOT + M^+PSS^- \qquad (1)$$

Here, M^+ represents a cation in the electrolyte and e^- is an electron. After the removal of gate bias, cations will come return to the electrolyte and the resistance of the PEDOT:PSS channel will recover, allowing the OECT to work stably. Owing to this important characteristic, drain–source current can be tuned by varying the gate voltage, which is the foundation of the chemical and biological sensing properties of OECT, as shown in Fig. 1c (transfer curve). In the glucose assay, when glucose solution is in contact with the OECT device the enzymatic reaction between glucose and GOx immobilized on the gate electrode produces hydrogen peroxide (Fig. 2a). After this, electrochemical oxidation of the hydrogen peroxide is catalyzed by Pt NPs on the gate electrode under a positive bias. The oxidation process results in the transfer of

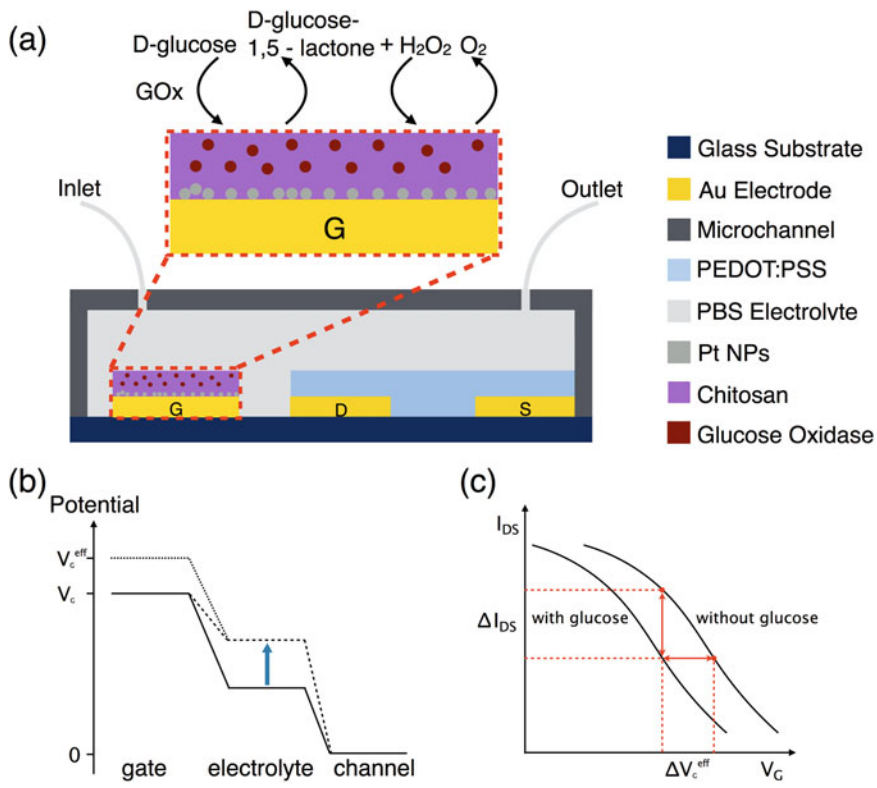

Fig. 2 (**a**) Schematic diagram of OECT-based glucose sensor integrated with microfluidic channel and enzymatic reaction on gate electrode. (**b**) potential distribution change after glucose injection in gate–electrolyte and electrolyte–channel interface. (**c**) relationship between drain–source current change and effective gate voltage shift after glucose injection

electrons to the gate electrode and changes the electrical double layer at the gate–electrolyte interface, thereby decreasing the voltage drop at the gate–electrolyte interface and increasing the potential applied to the active channel, as well as the corresponding effective gate voltage V_G^{eff} of the transistor (Fig. 2b) [4], The relationship is given by:

$$V_G^{eff} = V_G + (1+\gamma)\frac{\kappa T}{2q}\ln[H_2O_2] + \text{constant} \qquad (2)$$

where γ, the ratio between the capacitance of two interfaces (electrolyte–channel interface (C_c) and electrolyte–gate interface (C_G)) is given by $\gamma = C_c/C_G$, κ is the Boltzmann constant, and T is temperature in °C. The existence of glucose solution in the OECT device increases the effective gate voltage and causes a negative shift of the drain–source current of the device as shown in Fig. 2c. By monitoring the drain–source current decrease of the device we can obtain the effective gate voltage change from the transfer curve and determine the glucose concentration in the analyte.

Here we introduce the method of fabricating an OECT-based glucose sensor including the immobilization of GOx and a bias-free two-step dip coating method for deposition of Pt NPs.

2 Materials

2.1 Electrodes Deposition

1. Glass slide (Gold Seal).
2. Diamond glass cutter (nickel plated, pkg of 1 ea, Sigma-Aldrich).
3. Stencil shadow mask.
4. Ultrasonic cleaner (Branson, 2800).
5. Plasma cleaner (Harrick Plasma).
6. Thermal evaporation chamber (Minibox Conversion Moorfield).
7. Atomic force microscope (AFM, Multimode8-U, Bruker).

2.2 Active Channel Preparation

1. Poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS) (PH 1000, Clevios) was stored under 4 °C in refrigerator.
2. (3-glycidyloxypropyl) trimethoxysilane (GOPS) (≥98%, Sigma-Aldrich).
3. 4-Dodecylbenzenesulfonic acid (DBSA) (mixture of isomers, ≥98%, Sigma-Aldrich).
4. Ethylene glycol (EG) (99+%, International Laboratory USA).
5. Syringe filter (PTFE, 0.45 μm pore size, Hong Kong Labware Co. Ltd.).
6. Kapton tape (DuPont, USA).
7. Magnetic stirrer bar (Azlon, U.K.).
8. Spin coater (SC 100, Weinview).

2.3 Enzyme Immobilization

1. Phosphate buffered saline (PBS) (10× concentrate, BioPerformance Certified, suitable for cell culture, Sigma-Aldrich).
2. Glucose oxidase from aspergillus niger (GOx) (Type II, ≥15,000 units/g solid, without added oxygen, Sigma-Aldrich) was stored in −20 °C.
3. Chitosan (CHIT) (Medium molecular weight, Sigma-Aldrich) was stored in dry box under humidity 25%.
4. Acetic acid (100%, AnalaR).
5. Teflon tape (Lohmann Technologies UK Ltd).

2.4 PVP-Capped Pt NPs Deposition

1. Conditioner (ML-371, Rockwood Electrochemicals Asia Ltd).
2. Chloroplatinic acid hexahydrate ($H_2PtCl_6{\cdot}6H_2O$) (ACS reagent, ≥37.50% Pt basis, Sigma-Aldrich).
3. Polyvinylpyrrolidone (PVP) (M.W.8000, Acros Organics).
4. Sodium borohydride ($NaBH_4$) (powder, ≥98%, Sigma-Aldrich).
5. UV-Ozone cleaner (Jelight Company Inc.).
6. Hot plate (C-MAG HS7 digital, IKA).

2.5 Microfluidic Channel Fabrication

1. Polydimethylsiloxane (PDMS) precursor (Sylgard 184 Silicone Elastomer, Dow Corning).
2. Curing agent (Sylgard 184 silicone elastomer curing agent).
3. SU-8 photoresist (Gersteltec).
4. Silicon wafer (University Wafer, USA).

2.6 Glucose Detection

1. D-(+)-Glucose (≥99.5%, GC, Sigma-Aldrich).
2. SourceMeter (Keithley 2602A).
3. Electrical probes (Cascade, Microtech).
4. Silver paste (Ted Pella, INC).
5. Micro medical tubing (I.D. = 0.38 mm, O.D. = 1.09 mm, Scientific Commodities INC.).
6. Syringe pump (LongerPump).

3 Methods

3.1 Experimental Setup

Figure 3 shows the experimental setup of using OECT as a glucose sensor. A SourceMeter was used to provide gate–source and drain–source voltage to drive the device, and three electrical probes were used to connect the drain, source, and gate electrodes. The electrodes were coated with silver paste to improve electrical contact. Micro medical tubing was used to connect the inlet and outlet of the glucose sensor. A syringe pump was used to inject the glucose solution into the microfluidic channel for detection. An enlarged view of the glucose sensor device is shown in the insert of Fig. 3. The length and width of the sensor is 4.5 cm and 2.5 cm respectively. When using the OECT for glucose sensing it is very important to properly modify the gate electrode. We deposited Pt NPs on the gate electrode to improve the sensitivity, followed by immobilization of GOx using the biocompatible polymer chitosan.

3.2 Synthesis of PVP-Capped Platinum Nanoparticles

1. 1 g $H_2PtCl_6.6H_2O$ precursor was dissolved in 200 mL deionized water under stirring for for 5 min.
2. 500 mg PVP with a molecular weight of 8000 was dissolved in this solution and used as a capping layer.

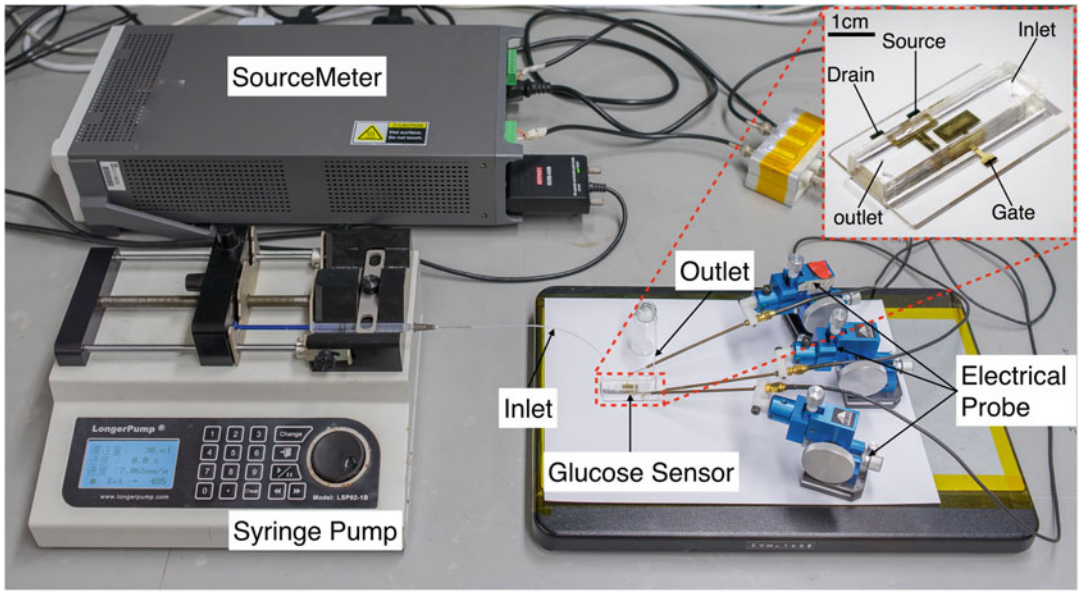

Fig. 3 Experimental setup of OECT-based glucose sensor for glucose sensing

3. Reductant sodium borohydride solution (0.125 M) was prepared in deionized water and gradually added into the above solution under stirring until color of the whole solution changed from brown to black, which indicates the formation of PVP-capped platinum nanoparticles.

3.3 Electrode Deposition

1. Glass slide was cut to 25 mm × 45 mm using a diamond cutter (**Note 1**).
2. Glass slide was rinsed in deionized water and sonicated in ultrasonic cleaner for 5 min, followed by cleaning with acetone and 2-propanol under identical conditions.
3. Repeat step two again and store the glass slide in 2-propanol for thermal annealing at 250 °C until boiling.
4. Glass slide was dried under a stream of nitrogen gas.
5. Glass slide was cleaned with oxygen plasma using a plasma cleaner for 15 min.
6. Glass slide was put into a thermal evaporation chamber for metal deposition (**Note 2**).
7. 100-nm-thick gold drain, source, and gate electrodes were deposited on glass substrate through a stencil shadow mask (Fig. 4a) which is fabricated by Photo-Chemical Machining (PCM). Before gold deposition, a thin layer of Cr with a thickness around 10 nm was deposited to improve the adhesion between Au electrode and substrate. Evaporation pressure was around 2×10^{-6} Pa and the evaporation rate for Au and Cr are

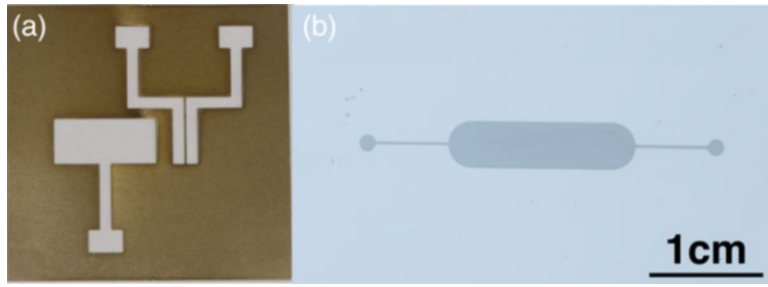

Fig. 4 Photograph of (**a**) stencil shadow mask and (**b**) mode of microfluidic channel

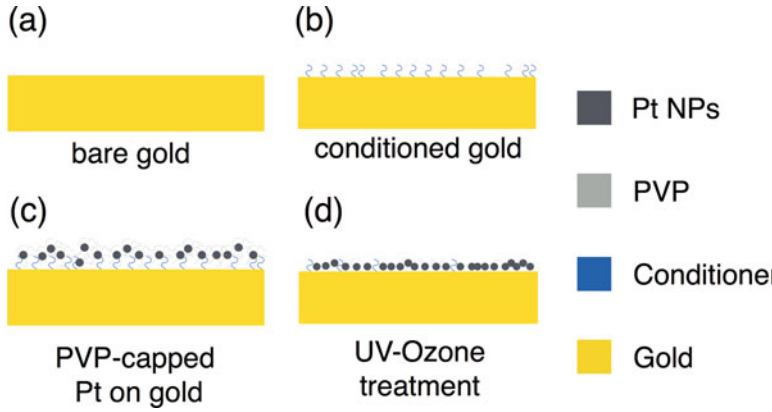

Fig. 5 Scheme of Pt NPs deposition by bias free two-step dip coating method

2 Å/s and 0.5 Å/s respectively. The deposition time for Au and Cr was around 10 min and 3 min respectively. The thickness of Au and Cr was measured by atomic force microscope.

3.4 Gate Electrode Modified by Pt NPs

1. After thermal evaporation, the glass slide with electrode was cleaned using the same procedure as described in Subheading 3.3.
2. Kapton tape was used to cover the whole glass slide except for the gate electrode surface.
3. The glass substrate with exposed gate electrode was cleaned by UV-Ozone cleaner for 10 min.
4. A bias free two-step dip coating method was used to deposit Pt NPs on the gate electrode (Described in following text) [5]. Conditioner was diluted to 1% (v/v) by deionized water and heated to 60 °C on a hot plate.
5. PVP- capped Pt NPs solution was heated to 40 °C.
6. The cleaned glass slide was immersed in 1% conditioner for 5 min (Fig. 5a, b) followed by immersion in PVP-capped Pt NP solution for 5 min. (Fig. 5c) Before each step, the substrate is rinsed by DI water and sonicated for 3 s to remove any residue.

After the dip coating process, the glass slide was dried by nitrogen gas (**Note 3**).

7. Post treatment of the substrate is carried out by UV-Ozone for 15 min. (Fig. 5d) This process is used to remove the conditioner on the gate electrode underneath the Pt NPs and the PVP capping layer to enhance the contact between the Pt NPs and gate electrode and also the active surface area of Pt NPs.

3.5 Active Channel Preparation

1. Teflon tape was used to cover gate surface and Kapton tape was used to cover other areas to expose channel area of the device.
2. Glass slide with exposed channel area was cleaned with UV-Ozone cleaner for 5 min.
3. 2 mL PEDOT:PSS was mixed with 100 μL ethylene glycol, 5 μL DBSA and 1 wt% GOPS [6] followed by stirring for 1 h at 1000 rpm.
4. Mixed PEDOT:PSS solution was filtered using a PTFE filter with hole diameter 0.45 μm.
5. Three drops of filtered PEDOT:PSS solution was placed on active channel surface and spin-coated at 2500 rpm for 65 s. Spin acceleration was set to 500 rpm/s and the acceleration time is 5 s.
6. Covered Kapton tape and Teflon tape was removed and the device was thermally annealed at 140 °C under N_2 atmosphere for 1 h (**Note 4**).

3.6 Enzyme Immobilization

1. Chitosan (CHIT) solution was prepared by dissolving CHIT (50 mg) in acetic acid solution (10 mL, 50 mM) and followed by electromagnetic stirring for 1 h at 1000 rpm.
2. Glucose oxidase (GOx) (15 U/mg) stock solution was prepared by dissolving 30 mg GOx in 2 mL PBS solution and followed by electromagnetic stirring for 1 h at 1000 rpm.
3. 1 mL GOx solution was mixed with 1 mL CHIT solution and followed by sonication for 20 min before use.
4. The device was rinsed with deionized water to remove any residues, Teflon tape was used to cover channel surface and Kapton tape was used to cover other areas to expose gate area. The device with exposed gate area was put into UV-Ozone cleaner for 5 min (Fig. 6a).
5. Covered Kapton tape and Teflon tape were removed and 10 μL GOx/CHIT mixture solution was placed on the Au/Pt NPs gate electrode (Fig. 6b) and dried at 4 °C in a refrigerator overnight (Fig. 6c).
6. After drying of CHIT/GOx film, the device was rinsed in deionized water to remove any non-immobilized enzyme.

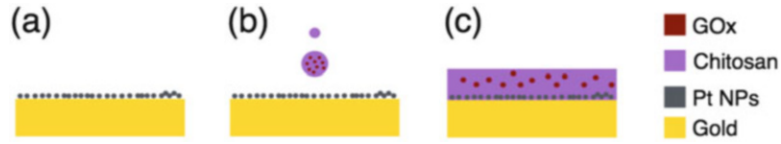

Fig. 6 Scheme of GOx immobilization on gate electrode of OECT-based glucose sensor

3.7 Integration with Microfluidic Channel

1. SU-8 photoresist was used to form mode of microfluidic channel by conventional soft lithography process on a silicon substrate as shown in Fig. 4b.
2. PDMS precursor and a curing agent were mixed at a weight ratio of 10 to 1.
3. PDMS mixture was stirred for around 10 min followed by degassing in vacuum oven for ~15 min.
4. Degassed PDMS mixture was poured on the silicon substrate with SU-8 mode and heated in a furnace at 90 °C for 1 h.
5. Cured PDMS channel with a rectangular cross section (thickness 50 μm, width 4.5 mm) was peeled off from the silicon substrate, cut and punched to connect micro tubes.
6. Schematic diagram of PDMS channel fabrication process is shown in Fig. 7.
7. PDMS channel and as-prepared OECT device was bonded by oxygen plasma treatment for about 3 min (**Note 5**).

3.8 Device Characterization and Glucose Detection

After fabrication of the OECT-based glucose sensor, measurement of the device was carried out with the setup shown in Fig. 3. PBS was injected into microfluidic channel and used as electrolyte for characterization. A typical transfer curve of the device is shown in Fig. 8a. For glucose sensing, glucose solutions of different concentrations were injected into the microfluidic channel at a flow rate 2 μL/s and a total injection volume 30 μL. After glucose injection the channel current decreased. In order compare channel current decrease caused by different glucose solutions, normalized current (NC) was calculated according to the following equation:

$$NC = \left| \frac{I_D^{Conc=c}}{I_D^{Conc=0}} \right| \tag{3}$$

where $I_D^{Conc=0}$ and $I_D^{Conc=c}$ represent channel currents before and after injection of glucose at the concentration of interest, respectively. As shown in Fig. 8b, glucose solution with concentration as low as 0.1 μM caused an obvious decrease of NC compared with the control sample (PBS solution only). At the same time, four single tests were conducted and showed negligible differences between tests, confirming the reliability of our glucose sensor.

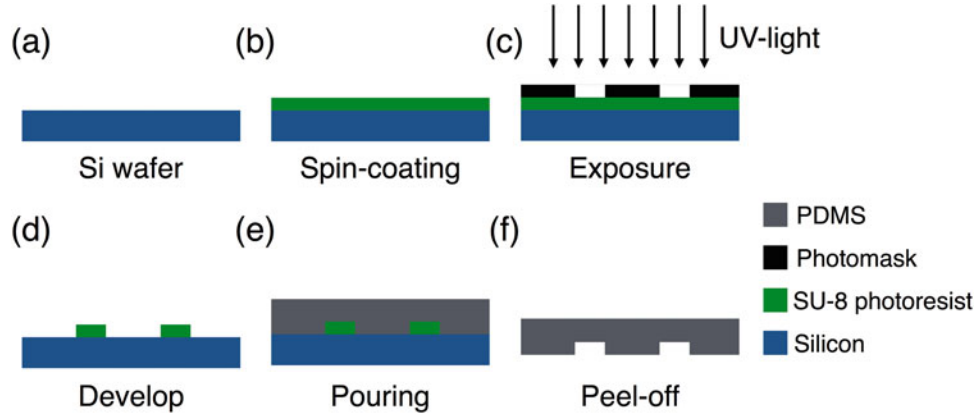

Fig. 7 Schematic diagram of PDMS-based microfluidic channel fabrication process. (**a**) Cleaning of silicon wafer. (**b**) Spin-coating SU-8 photoresist. (**c**) UV-exposure through a photomask. (**d**) Developing the photoresist to make needed patterns. (**e**) Pouring PDMS and followed by thermal annealing. (**f**) Peeling off the PDMS to form microfluidic channel

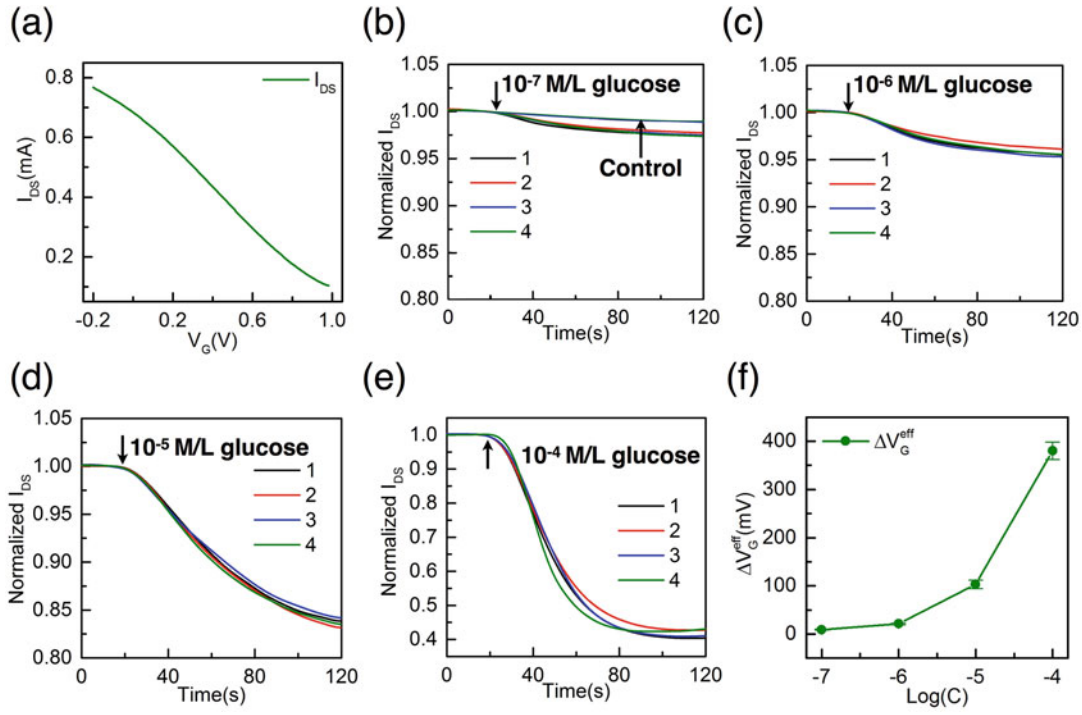

Fig. 8 (**a**) Transfer curve of OECT-based glucose sensor. V_{DS}=−0.2 V. (**b**) comparison between normalized current (NC) after injection of PBS control solution and 10^{-7} M glucose. (**c**) NC after injection 10^{-6} M glucose solution. (**d**) NC after injection 10^{-5} M glucose solution. (**e**) NC after injection 10^{-4} M glucose solution. (**f**) relationship between effective gate voltage shifts and glucose concentration. For NC vs. time curves, V_{DS} was fixed at −0.2 V and V_G was fixed at 0.5 V

Larger decreases of NC can be obtained by further increasing the glucose concentration to 1 μM, 10 μM and 100 μM as shown in Fig. 8c–e. Effective gate voltage shift ΔV_G^{eff} cause by the glucose solution can be calculated and is shown in Fig. 8f. A detection limit of 10^{-7} M can be obtained in our device (**Notes 6** and **7**).

4 Notes

1. Tweezers need to be cleaned in the clean step of glass slide preparation to avoid contamination when using tweezers to clamp the glass slide for drying under nitrogen gas.
2. During thermal evaporation of Cr and Au, the area on the plate that attaches to the glass slide was first put on top of the Cr target. After Cr deposition, spin the plate so that it located on top of the Au target. This can help align the top Au electrode and bottom Cr adhesion layer.
3. Conditioner and PVP-capped Pt NPs solution can be reused after Pt NPs deposition.
4. In the process of thermal annealing of the PEDOT:PSS active channel, a glass petri dish should be used to cover the glass substrate.
5. When aligning the OECT device with the microfluidic channel during the bonding process, the channel area is more visible when placed over a black tape, making alignment easier.
6. Detection of glucose fails mostly due to the leakage of the microfluidic channel. If the bonding between the microfluidic channel and OECT device is not strong enough, leakage of electrolyte will always happen. This bonding problem is usually due to insufficient cleaning of the OECT surface. A protective layer of parylene can be deposited on the OECT just after the gate, drain and source electrode deposition. Plasma etching can be used to remove the parylene layer on the gate and channel area. After completion of the fabrication process, the parylene layer can be peeled off to ensure the surface cleanness of OECT for bonding with the microfluidic channel.
7. During the glucose sensing process, after detection of a glucose sample the solution in the microfluidic channel of the device should be removed by air and washed by PBS solution for about 3 min using a flow rate 2 μL/s.

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