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Heat-transfer-printed Glucose Biosensor for Use in Diapers

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Wearable biosensors that can monitor health conditions are currently attracting attention. In this study, we investigated a method to fabricate a glucose biosensor by heat transfer printing. A screen-printed glucose oxidase-modified electrode formed on a polyethylene terephthalate (PET) substrate was transferred onto a nonwoven fabric. Conditions for thermal transfer while retaining enzyme activity were investigated, and electrochemical evaluation of the glucose biosensor was conducted. Enzyme-catalyzed reactions were found to readily occur on the electrode surface after heat transfer printing. A linear relationship was obtained in the glucose concentration range from 0.1 to 20 mmol dm^{-3}. In general, the glucose concentration in urine is 3–6 mmol dm^{-3} in healthy persons and 7 mmol dm^{-3} or higher in diabetics. This fabrication method is expected to be widely applicable to future biosensors for monitoring urine sugar in diapers and other products.

1. Introduction

Urine glucose biosensors have attracted attention for their application in the management of diabetes mellitus, whose incidence has been rapidly increasing in recent years.[1–4] Urine glucose biosensors aid the management of diabetes in diabetes patients and could also enable its prevention by facilitating an easy and non-invasive measurement of urine glucose concentration (hereinafter referred to as "urine glucose"). Urine glucose is a more reliable indicator of an individual's glycemic status than blood glucose levels. It is known, for instance, that in postprandial hyperglycemia, blood glucose levels rise rapidly only immediately after a meal and return to normal over time. Therefore, conventional fasting blood tests cannot detect abnormal levels of glucose in the body, and diabetes may progress undetected and become severe by the time symptoms are recognized. A sensing device that can detect urine glucose and monitor its concentration on a regular basis in a simple manner will allow the early detection and prevention of diabetes mellitus.

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In most previous studies on urine glucose sensors, the sensors existed independently (that is, unattached to the subject) and the subject manually collected the urine sample and measured its glucose concentration by the dropwise addition of a specific volume of the urine on the sensor or immersing the sensor in the urine sample.[1–4] However, there has recently been an attempt to measure urine glucose without collecting urine by installing a sensor in a wearable device.[5–8] This is intended for people who need nursing care, and aims at enabling the easy monitoring of urine glucose by allowing the direct evaluation of urine by simply wearing the specific device. In this context, a self-powered glucose biosensor system that can be integrated into diapers (i.e., wearable) to detect the urine glucose of diabetes patients was previously developed[9] by applying screen-printing technology using paper as a substrate. However, such diaper-mounted urine glucose sensors require preprocessing to mount the sensor stably in the diaper to integrate it into the diaper.

In the present study, to facilitate the integration of glucose sensors into diapers, we developed a glucose sensor system by using a novel heat transfer printing process, wherein heat and pressure are applied to form a glucose biosensor on a nonwoven fabric surface. This printing method enables printing on a wide variety of objects, regardless of the shape or material. After the glucose sensor is formed, the sensor is heat-transferred to the nonwoven fabric used for diapers, which enables the sensor to be stably embedded in a precise position. On the other hand, applying heat or pressure to enzymes or mediators may cause denaturation or inactivation. In this study, we measured the response of the glucose sensor before and after transfer and performed a basic evaluation of whether the sensor could be used as a urine glucose sensor in the future.

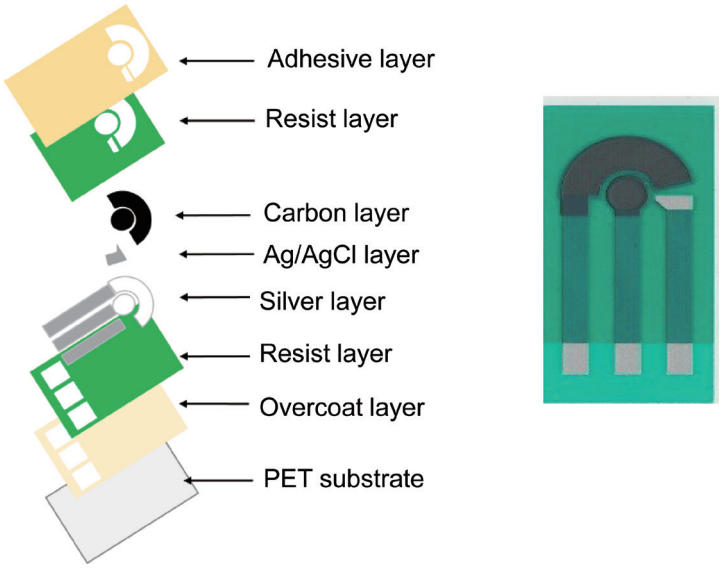
2. Materials and Methods

2.1 Materials

A screen printer (LS-150TV, Newlong Seimitsu Kogyo Co., Ltd.) was used to fabricate a glucose biosensor. A release agent-treated polyethylene terephthalate (PET) film, an overcoat ink, and an adhesive ink were purchased from Japan Polymark Co., Ltd. A resist ink (S-40 C518) was purchased from Taiyo Ink Mfg. Co., Ltd. A silver ink (SAP-40FL) was purchased from Sanwa Chemical Industry, Japan. A carbon ink (JELCON CH-8) was purchased from Jujo Chemical. Glucose oxidase (GOD, from *Aspergillus niger*, 100 U/mg) was purchased from Fujifilm-Wako Chemicals. Tetrathiafulvalene (TTF, 98% purity) was purchased from Tokyo Kasei Kogyo.

2.2 Fabrication of three-electrode chip on PET substrate

Figure 1(a) illustrates the fabrication of a three-electrode chip on a PET film substrate for the creation of the glucose biosensor. First, an overcoat layer was printed on the substrate and dried at 50 °C for 10 min. Next, a resist layer was printed as a protective layer and dried at 120 °C for 60 min, and then Ag layers were printed as the conducting wires and dried at 130 °C for 30 min.



(a) (b)

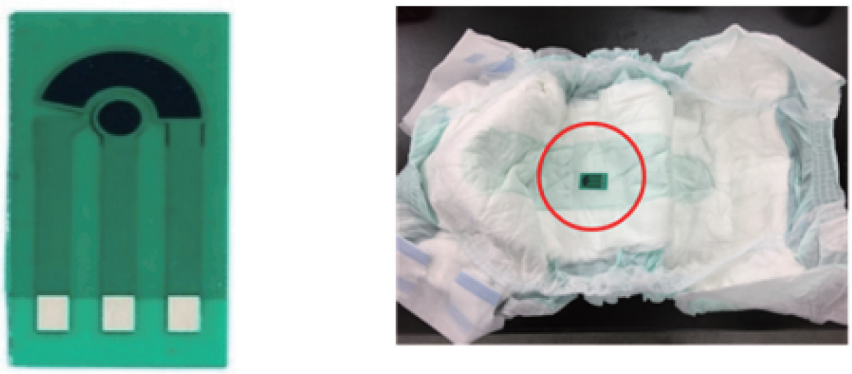
Fig. 1. (Color online) (a) Schematic illustration of the fabrication process and (b) photograph of the three-electrode chip used in the glucose biosensor.

In addition, a carbon layer was printed to function as the working electrode (WE) and counter electrode (CE) and dried at 120 °C for 12 h. A Ag/AgCl layer was printed using Ag/AgCl ink, in which Ag ink and AgCl were mixed at a ratio of 10:1. The Ag/AgCl served as the reference electrode (RE). Another resist layer was then printed to protect the conducting wires. Finally, an adhesive layer was printed on top of this resist layer. The electrode area was 0.196 cm^2 for the WE, 0.735 cm^2 for the CE, and 0.09 cm^2 for the RE. Six types of screen-printing plates manufactured by Mitani Micronics, Inc. were used. For the squeegee, an E-Fine squeegee (Newlong Seimitsu Kogyo Co., Ltd.) with a hardness of 70° was used. The angle of attack was 75°. The coating and printing speeds were 30 mm/s and the clearance length was 1.13 mm. The fabricated three-electrode chip (electrode before transfer) is shown in Fig. 1(b).

2.3 Fabrication of glucose biosensor on nonwoven fabric

An enzyme-modified electrode was prepared on the WE of the three-electrode chip prior to heat transfer printing. First, 2.5 µL of TTF saturated in methanol solution was cast and dried for 10 min, then 2.5 µL of 1 mol dm^{-3} phosphate buffer containing GOD (10 U/1 µL) was modified by dropwise addition of the solution on the electrode surface and drying for 15 min.

A temperature of 120 °C and a pressure of 0.85 MPa were applied for 10 s from the top of the enzyme-modified electrode chip using a thermal transfer machine (Japan Polymark Co., Ltd.) to fabricate a glucose biosensor on a nonwoven fabric. The adhesive layer was melted by heat to bond the nonwoven fabric and the three-electrode chip. Finally, the PET film was peeled off from the overcoat layer to form the glucose biosensor on the nonwoven fabric. Figure 2(a) shows a photograph of the glucose biosensor formed on the nonwoven fabric. Figure 2(b) shows a photograph of the biosensor mounted on a diaper.



(a) (b)

Fig. 2. (Color online) Photographs of the heat-transfer-printed glucose biosensor chip on (a) nonwoven fabric and (b) diaper.

Through this process, the sensor is integrated with the nonwoven fabric. Therefore, none of the sensor directly touches the human skin when mounted on a diaper. On the other hand, the overcoat layer peels off from the PET film during the heat transfer, so that the leads and the electrochemical measurement device can be connected from the top of the sensor without passing through the paper.

2.4 Electrochemical measurements

Cyclic voltammetry was performed using samples with various glucose concentrations to confirm the concentration dependence of the current density generated by the glucose biosensor. The measurements were performed by dropwise addition of the sample onto the electrode through the paper. Here, 500 μL of 1 mol dm^{-3} phosphate buffer (pH 7) containing glucose at concentrations of 0.1, 1, 3, 5, 7, 10, 20, 25, 50, 75, and 100 mmol dm^{-3} was used as the electrolyte. The scanning speed was 10 mV s^{-1}. All measurements were performed in triplicate. Error bars were determined using Student's t distribution at a 68.3% confidence level (n = 3).

3. Results and Discussion

Figure 3 shows cyclic voltammograms of the glucose biosensor before and after heat transfer printing. Clear catalytic waves were observed for both electrodes at potentials higher than −0.15 V, indicating an increase in the catalytic oxidation current of glucose. The maximum current densities were 265 and 365 μA cm^{-2} before and after heat transfer printing, respectively. In the glucose biosensor, glucose is converted to gluconic acid ($C_6H_{11}O_7$) by GOD, and TTF^+ is converted to TTF by electron transfer from the reduced GOD. When a constant voltage is applied to the electrode, the resulting TTF donates an electron to the WE and reverts to its ionic state (TTF^+). The amount of glucose presented in the sample can be determined by measuring the current generated at this point.

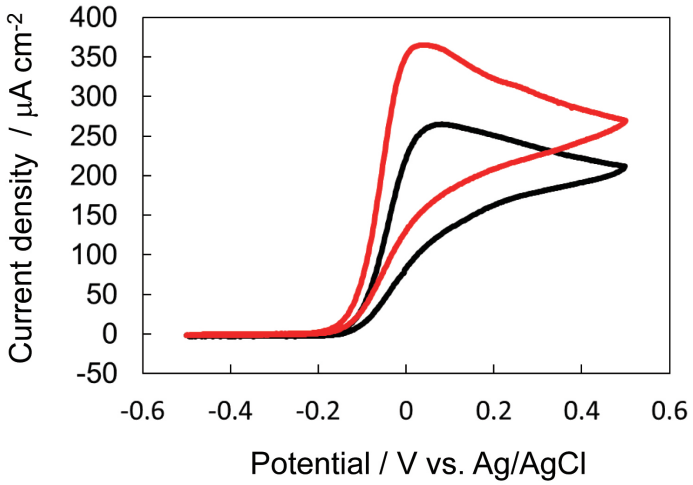


Fig. 3. (Color online) Cyclic voltammograms of the glucose biosensor. Readings were taken using 1 mol dm^{-3} phosphate buffer in the presence of 100 mmol dm^{-3} glucose before (solid black curve) and after (solid red curve) heat transfer printing.

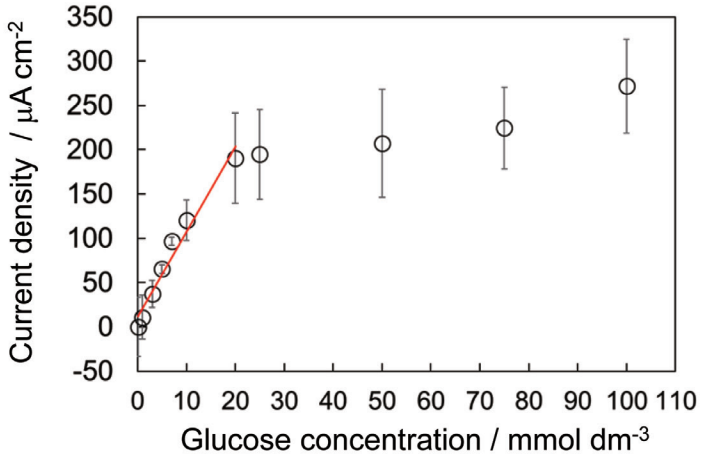


Fig. 4. (Color online) Correlation between current density of glucose biosensor and glucose concentration of sample. Error bars were determined using Student's t distribution at 68.3% confidence level ($n = 3$).

The cyclic voltammogram of the heat-transfer-printed glucose biosensor shows that its maximum current density was greater than that of the pre-transfer glucose biosensor. The TTF used as a mediator is partially crystallized because it is supersaturated. The thus formed crystals are densely populated on the electrode surface, and the heat and pressure applied during the transfer of the biosensor pulverize and melt the crystal particles, making TTF^{+} react more easily.

A calibration curve was prepared by plotting of the maximum current density observed in cyclic voltammograms with different concentrations of glucose solutions (Fig. 4). A linear relationship was obtained between glucose concentrations of 0.1 and 20 mmol dm^{-3}.

Diabetes is diagnosed when the measured fasting blood glucose level is 126 mg/dL (7.0 mM) or higher in at least two separate tests.[10] Since the glucose biosensor developed in this study shows linearity and reproducibility in the concentration range used for the determination of diabetes, it can be used for the diagnosis and monitoring of diabetes.

4. Conclusions

A new glucose biosensor that can be directly mounted on a diaper was created. A three-electrode chip was fabricated by layering ink on a PET film substrate treated with a release agent using screen printing. The three-electrode chip was then transferred to the diaper fabric using a heat transfer system. The activity of the enzyme at high temperatures was investigated, and it was confirmed that the enzyme activity was retained, indicating that the enzyme did not undergo thermal deactivation. Electrochemical measurements before and after heat transfer printing showed enzyme-catalyzed oxidation currents at potentials higher than −0.15 V in both cases, and the maximum current density was higher following transfer than before transfer. Moreover, a calibration curve was plotted for the glucose biosensor by plotting the average maximum current density against the corresponding glucose concentration. A linear relationship was obtained for glucose concentrations in the range of 0.1 to 20 mmol dm^{-3}, confirming the potential commercial application of this glucose biosensor for urine glucose detection.

In the near future, we would like to evaluate the device in weakly acidic solutions containing uric acid and sodium chloride, such as artificial urine, and to conduct mounting evaluation tests using the device in diapers.

Acknowledgments

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