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Simultaneous Electrochemical Measurement using Paper Fluidic Channel on CMOS Chip

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Abstract

This paper described the new system of biosensing using CMOS chip. The system was expected to be used in various circumstances because it was suitable for miniaturization compared to the conventional system. To conduct electrochemical measurements, the new system used paper fluidic channel set on the CMOS chip to transport solution to the on-chip electrodes. The materials of paper fluidic channel were only paper and silicone resin, and these were biocompatible. In experiment, we carried out simultaneous detection of glucose and ethanol in liquid sample solutions on the 5mm square CMOS chip and paper fluidic channel. Furthermore, this system can detect various target molecules in addition to glucose and ethanol, and increase number of simultaneous measurement by adding some more process to the paper and CMOS chip.

Keywords*: CMOS biosensor chip, chromatography paper, paper fluidic channel, glucose, ethanol*

1. Introduction

In recent years, the aging of developed countries' society has caused increase in medical costs. To resolve this problem, point-of-care testing (POCT) has gathered attention. The CMOS circuits aimed to biochemical sensors have been studied for several decades [1]. As a method of POCT, biosensing using CMOS chip has been studied. The CMOS integrated circuits offer low cost, accurate, and real time biosensing without using external electrochemical system [2]. Various CMOS biosensors, such as gas sensor [3, 4], cancer marker sensor [5,6], and glucose sensor [7] have been demonstrated. In the conventional studies, electrodes and micro fluidic channel are set on the CMOS chip, sample solution is transported to electrodes by an external pump [2, 8]. However, in applying this method to POCT devices, there is such a problem that this method does not suit miniaturization of the entire system because of large pump attached externally to the sensor, and sample solution sometimes need to be filtrated before sensing. Meanwhile, a study of transporting solution in 3D microfluidic devices, using chromatography paper (ChrPr) patterned with SU-8 photoresist and stacking them by double sided adhesive tape, has been reported [9]. In this study, multiple analytes in the sample solution were detected simultaneously by color reaction induceded by reagents in the paper device. Under these circumstances, we aim to miniaturize entire system by integrating CMOS chip and biocomapatible ChrPr fluidic channel (ChrPr channel) that is made by adding some processing to ChrPr. Sample solution is transported to electrodes by ChrPr channel and be filtrated.

In this paper, we conducted simultaneous detection of glucose and ethanol in liquid sample by fabricating a structure to measure glucose and ethanol at the same time by integrating CMOS chip and ChrPr channel. This study is expected to contribute to developing biosensors based on a CMOS chip.

2. Research Method

To detect glucose and ethanol, enzyme and mediator must be fixed on the electrode. Figure 1 and the following equations describe the reaction scheme of glucose detection, where sufficient amount of glucose oxidase (GOD) and potassium ferricyanide (ferri) are immobilised on the electrode.

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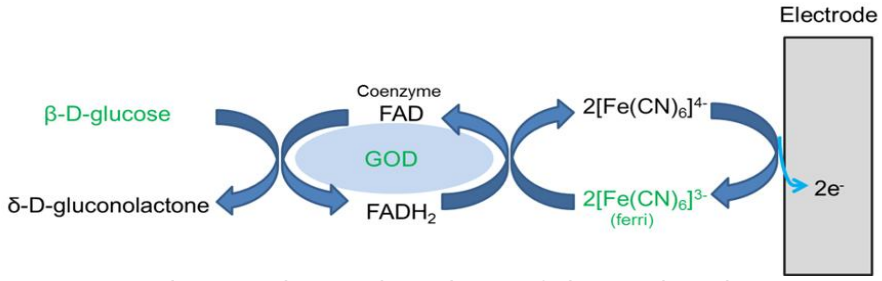

Figure 1. The reaction scheme of glucose detection

$$\beta\text{-D-glucose} + \text{GOD-FAD} \rightarrow \text{GOD-FADH}_2 + \delta\text{-D-gluconolactone}\,, \quad (1)$$

$$\text{GOD-FADH}_2 + 2[Fe(CN)_6]^{3-} \rightarrow \text{GOD-FAD} + 2[Fe(CN)_6]^{4-} + 2H^+\,, \quad (2)$$

$$2[Fe(CN)_6]^{4-} \rightarrow 2[Fe(CN)_6]^{3-} + 2e^-\,. \quad (3)$$

Figure 2 shows the reaction scheme of ethanol detection, where alchol oxidase (AOD), horse raddish peroxidase (HRP), and potassium ferrocyanide (ferro) are used.

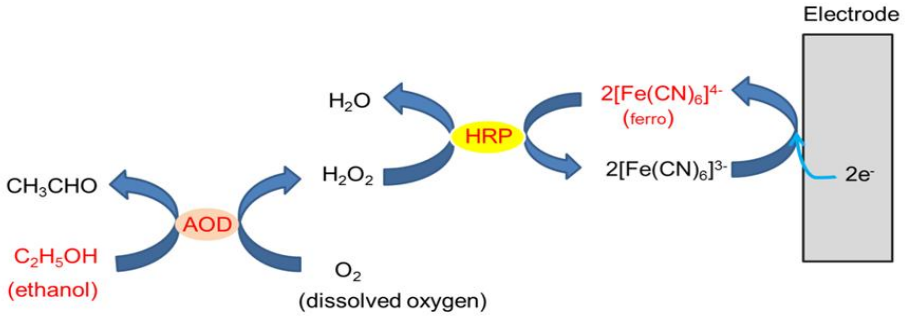

Figure 2. The reaction scheme of ethanol detection

The reactions are described by the following equations:

$$C2H5OH + O2 \rightarrow CH3CHO + H2O2, \quad (4)$$

$$H2O2 + 2[Fe(CN)6]^{4-} + 2H^+ \rightarrow H2O + 2[Fe(CN)6]^{3-}\,, \quad (5)$$

$$2[Fe(CN)6]^{3-} + 2e^- \rightarrow 2[Fe(CN)6]^{4-}. \quad (6)$$

Sample solution in this study is prepared from phosphate-buffered saline (PBS) as solvent since the optimum pH of GOD and AOD is around pH7.0.

Figure 3 shows device setup, where two channels were defined by attaching two rectangular ChrPr to square ChrPr having hydrophobic area. This ChrPr fluidic channel was set on the CMOS chip having electrodes on the surface. Dropped sample solutions are transported to electrodes without mixing with each other. One rectangular ChrPr includes ferri and GOD, and the other includes AOD and HRP. The sample solution intermingles with enzyme and mediator as it sinks into the ChrPr channel. As the sample solution reach to electrodes, electrical current derived from enzyme reaction shown in Figure 1 and 2 is measured. Ferro was separated from AOD and HRP to prevent undesirable chemical reaction before dropping sample solution so that background current unrelated to enzyme reaction is made small. We prepared sample solution of PBS, glucose, and ethanol. The PBS solution is unmodified PBS (0.01mol/l), pH at 25°C 7.0～7.4, 164-18541, Wako). The concentration of glucose in the solution is 10mM prepared from β-D-glucose (100953, MP Biomedicals,LLC) and PBS (0.01mol/l , pH at 25°C

7.0∼7.4, 164-18541, Wako). The concentration of ethanol in the solution is 1.0 V/V % prepared from ethanol (054-00464, Wako) and PBS (100mM pH=7.0).

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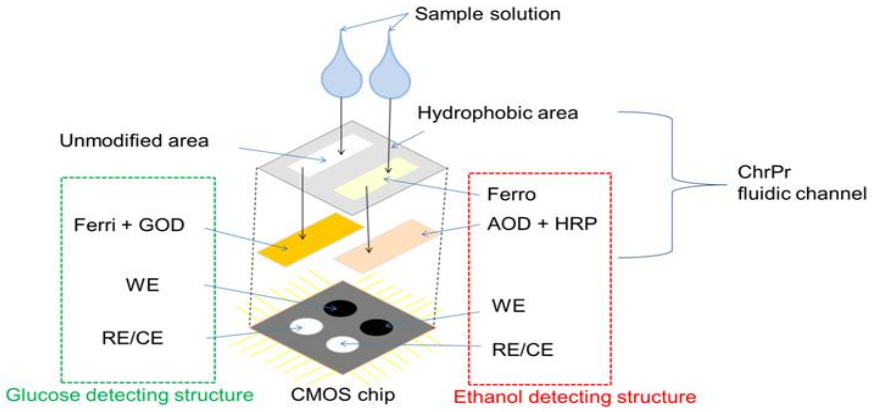

Figure 3. The schematic of device setup

The ChrPr channel has a two layer structure of upper and lower layer, and consists of ChrPr (1CHR, Whatman). Hydrophobic area is made by applying silicone resin (TSE3996-C, Momentive Performance Materials Japan), and then leaving it in room temperature until silicone completely solidifies. In defining the hydrophobic area, Robotic arm (Desktop Robot, SM200SX-3A, Musashi Engineering) and Dispenser (Precision dispensing machine, ML-5000X-, Musashi engineering) are used to apply desired amount of silicone resin to a desired position. As shown in Figure 4(a), silicone is applied to ChrPr so that two rectangular unmodified areas are in square hydrophobic area. After that, 0.5μL of ferro solution was dropped onto one side of hydrophilic area, the ChrPr is dried in an oven at 40°C for 15 minuets. The ferro solution contains ferro (165-03745, Wako) 30mM in PBS (100mM pH=7.0). After that, as shown in Figure 4(b), fabricating upper layer of channel is completed by cutting off outside of hydrophobic area. The lower layer is composed of two rectangular ChrPr. The size of lower layer's ChrPr is 5mm x 1.5mm. One of the two rectangular ChrPr is dipped into GOD + ferri solution, the other is dipped into AOD + HRP solution, and then these two ChrPr are dried in an oven at 40°C for 15 minuets. The AOD + HRP solution contains AOD (9073-63-6, SIGMA-ALDRICH) 70 units/mL and HRP (9003-99-0, Wako) 70 units/mL in PBS (100mM pH=7.0). The GOD+ferri solution contains ferri (161-03725, Wako) 35mM and GOD (074-02401, Wako) 1000units/mL in PBS (0.01mol/l, pH at 25°C 7.0∼7.4, 164-18541, Wako). In this paper, we call these ChrPr parts GOD+ferri paper, AOD+HRP paper. Figure 4(b) shows all parts of ChrPr channel. Attaching GOD+ferri paper to the unmodified area, and AOD+HRP paper to ferro area, silicone resin (TSE397-C) is applied to two sides to be fixed. In this way, ChrPr channel is completed (Figure 4(d)).

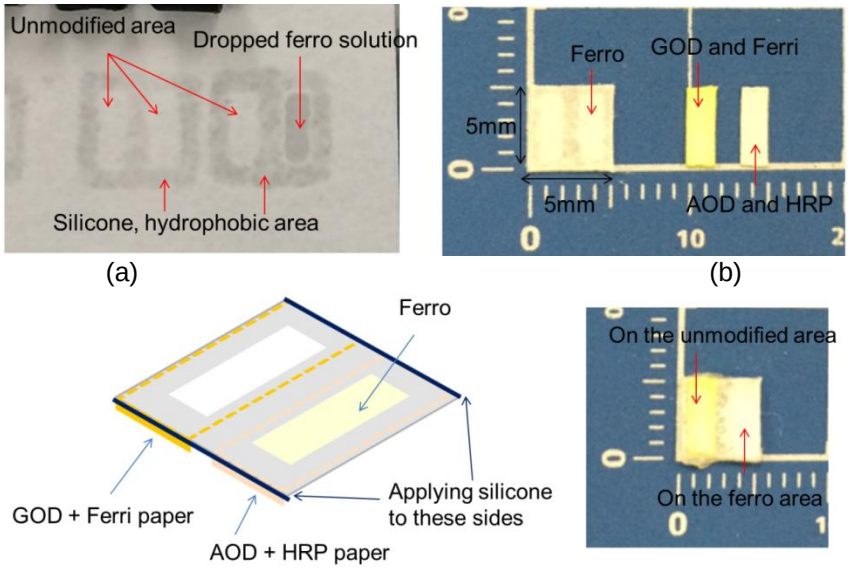

Figure 4. The steps of fabricating ChrPr channel. (a) The pattern of hydrophobic area, (b) All parts of ChrP channel, (c) The method of fixing parts, (d) Back of completed ChrPr channel

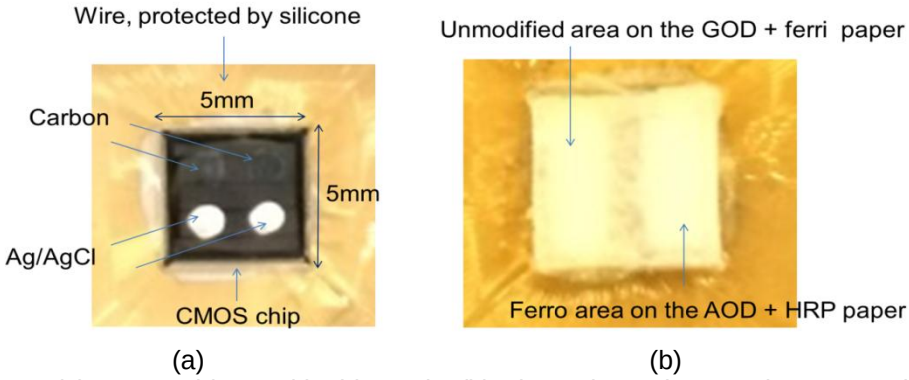

Figure 5. (a) CMOS chip used in this study, (b) ChrPr channel set on the CMOS chip

The CMOS chip (Rohm0.18μm 1P5M standard CMOS process) is designed in our laboratory, and has four metal pads on the surface. Wires in the CMOS chip are running so that all metal pads can be connected directly to external instrument. Two electrochemical measurements using two electrodes can be conducted simultaneously by making two sets of working electrode and reference / counter electrode (RE/CE) on the metal pads. The WE was formed by annealing the CMOS chip on hot plate at 300°C for 60 minutes after applying graphene ink (798983, Sigma-Aldrich) on to the metal pads. After that, RE/CE was formed by applying Ag/AgCl ink (011464, BAS) to the metal pads, and then drying ink in room temperature (25°C, humidity 60%) for 24 hours. After electrodes were formed, bonding wire was covered and protected by silicone resin (TSE 399-C) in order not to be disconnected.

The ChrPr channel was set on to the CMOS chip so that one set of WE and RE/CE contact to GOD + ferri paper, and another set of WE and RE/CE contact to AOD + HRP paper. The measurements were conducted using ALS/CH Instruments Electrochemical Analyzer (Model 6081E and Model 6108D, BAS). The initial potential between the WE and the RE/CE is 0.8V which is an adequate tage to induce oxidation reaction of ferro in the glucose detecting structure. That of the ethanol detecting structure is-0.6V to induce reduction reaction of ferri. In the case of glucose detection, it is estimated that ferri is induced and oxidation current appears

in the glucose detection area. In dropping ethanol solution, ferro should be produced and reduction current should appear in the ethanol detection area.

3. Results and Analysis

Dropping the sample solution, PBS, glucose solution, or ethanol solution, we conducted simultaneous detection of glucose and ethanol in the sample solution by chronoamperometry (CA) method. These measurements were intended to confirm that the target molecule is detected selectively in each area. The measurement is started 1 minute after dropping the sample solution (2.0μL) on both of the unmodified area and ferro area by the two ALS/CH Instruments Electrochemical Analyzer.

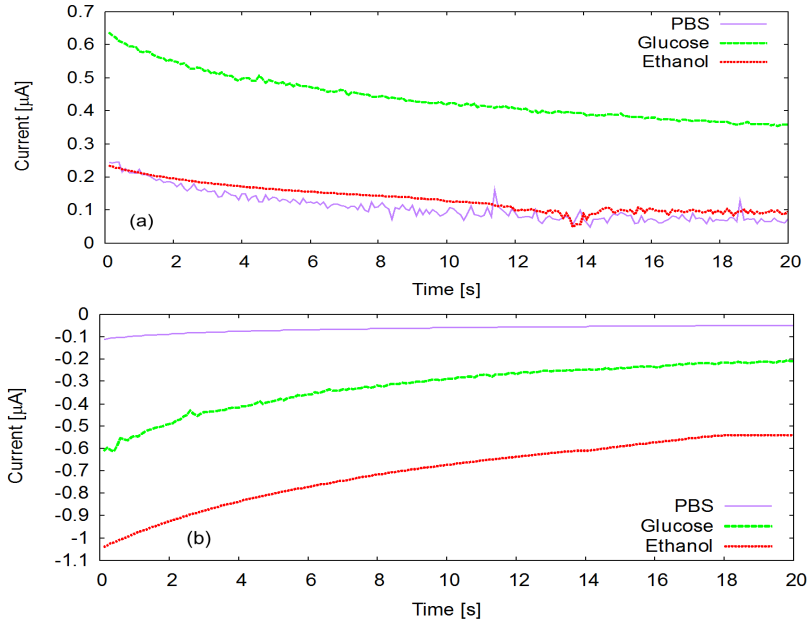

Figure 6. The result of simultaneous measurements, (a) Glucose sensing structure, (b) Ethanol sensing structure

Figure 6 shows the result of simultaneous measurements. Figure 6(a) shows the result of glucose sensing area. It indicates that the current in measuring glucose is very large compared to the case of PBS, whereas the current in measuring ethanol is approximately equal to that of PBS. The current in measuring glucose solution is estimated to originate from enzyme reaction induced by glucose shown in Figure 1. This means that glucose detecting structure detected glucose selectively. Figure 6(b) shows the result of ethanol sensing area, and indicates that the current level in measuring ethanol solution is much higher than that of PBS and glucose. This current is considered to be derived from ethanol, and therefore ethanol is considered to be detected specifically. From these results, proper operation of the sensing systems of detecting glucose and ethanol is confirmed.

4. Conclusion

This study showed a successful simultaneous and selective detection of glucose and ethanol on the 5 mm square CMOS chip integrating the ChrPr fluidic channel. Though we used GOD and ferri for detecting glucose, and used AOD, HRP, and ferro for detecting Ethanol in this study, in principle, we can change detection target by only changing enzyme and mediator. In the same way as this study, we can define 3D fluidic channel freely in the laminated ChrPr, making various pattern of hydrophobic area on laminated ChrPr [10]. Such ChrPr channel can

distribute dropped sample solution to electrodes evenly (Figure 7). In the future, this device is expected to be used as a sensor detecting many subjects in a sample solution at the same time.

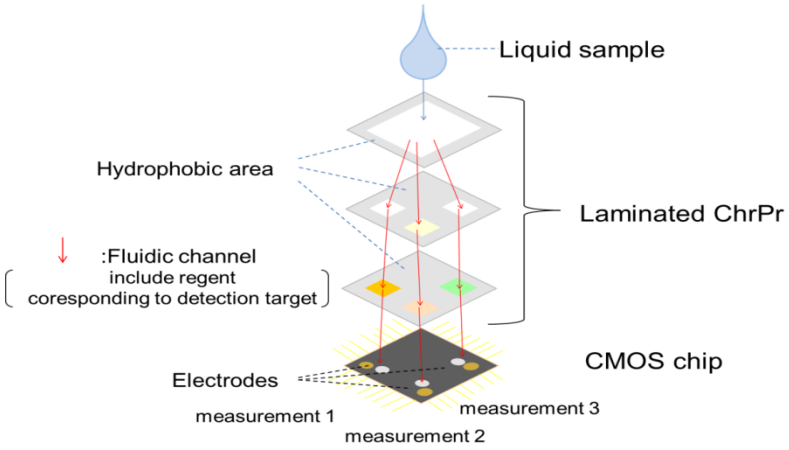

Figure 7. The schematic of further study

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