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Design and evaluation of the MOSFET type glucose biosensing system

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ABSTRACT

A health monitoring system (HMS) involving a blood extraction device with a new type of hybrid biosensor comprising an enzyme and a semiconductor has recently been developed. A MOSFET was used as the transducer. The gate electrode was extracted from the MOSFET using a cable. Gold (Au)-plate-immobilized glucose oxidase (Go) was used as a biosensor and attached to the gate electrode. Go was immobilized on a self-assembled spacer combined with an Au electrode by the cross-link method using BSA as an additional bonding material. The electrode could be used to detect electrons generated by the oxidization of hydrogen peroxide produced by the reaction between Go and glucose using the constant electric current measurement system of the MOSFET-type hybrid biosensor. The sensitivities for the diluted whole blood and blood plasma were 61.4 and 171.2 V/(mol/L), respectively. The hybrid biosensor was useful for HMS.

Keywords: MOSFET; micro-electro-mechanical systems; glucose biosensor.

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Introduction

Microfabrication technology, such as micro-electro-mechanical systems (MEMS), has been applied to the development of various healthcare-related products, including health monitoring systems (HMS) [**1-4**] and drug delivery systems [**5-7**]. As a combination of MEMS and biomedical systems, Bio-MEMS represent a particularly promising research field. The development of safe medical equipment based on Bio-MEMS technology for the human body is one of the most important and interesting research themes in the Bio-MEMS field. An important objective in HMS is the ability to check blood conditions in order to continuously monitor human health. Blood contains numerous health index markers, and it is very important to monitor these when health conditions are recognized. To sense lesions, we need to be able to detect specific markers. Therefore, biosensors that detect only specific markers are required [**8, 9**]. For example, in the case of diabetes mellitus, the determination of blood glucose levels is extremely important for diagnosis and effective management.

We have focused on the investigation of a compact and wearable HMS monitoring system that can detect blood glucose levels automatically in a diabetic patient. The device can extract a few microliters of blood through a painless microneedle using the negative pressure generated by an actuator in the blood tank, similarly to a female mosquito extracting human blood with muscle motion. The HMS consists of: (1) an indentation unit with a microneedle to generate the skin penetration force using a shape memory alloy (SMA) actuator; (2) a pumping unit (blood extraction device) using a bimorph PZT piezoelectric actuator to extract the blood; and (3) a biosensor-immobilized glucose oxidase (Go) to detect the amount of glucose in the extracted blood. There are few papers on wearable HMS monitoring systems, but all have separately discussed the glucose sensor, microneedle and pumping system.

We designed a novel hybrid biosensor using enzymes and semiconductors. The MOSFET was selected as a transducer, and a gold (Au)-plate-immobilized Go on a gate electrode was used as a biosensor [**9-14**]. The MOSFET is easy to design the system and combine the gold (Au)-plate-immobilized Go. To detect signals from the biosensor, the three-electrode method is used. We adopted the cross-link method using BSA as an additional bonding material to combine Go with a spacer immobilized by a self-assembled monolayer (SAMs) on an Au plate [**15-18**].

In this paper, the performance of this MOSFET-type hybrid biosensor system is evaluated. The hybrid biosensor performs glucose detection in the collected blood. The glucose concentration is identified by estimating the threshold voltage of the gate electrode. Finally, the hybrid biosensor is mounted in an HMS, the glucose solution is extracted and detected, and the signal is detected from the glucose biosensor. We also discuss a glucose monitoring system comprising a sensor, microneedle and actuator in the blood tank, as well as a pumping system similar to that present in a female mosquito.

Experimental

Materials

Recombinant Go (EC 1.1.3.4, lyophilizes) from *Aspergillus niger* was purchased from Roche Co. (Basel, Switzerland). A Pt plate of 1.0 × 1.0 × 0.1 mm used as the extracted electrode in MOSFET was purchased from Nagai Metal Kogyo (Osaka, Japan), and its purity was 99.95%. The spacer used was cystamine hydrochloride from Wako Pure Chemical Industries (Osaka, Japan). The cross-linking agent, glutaraldehyde, from Wako Pure Chemical Industries was used as a 25% solution (Osaka, Japan). All the other chemicals used were commercial products of the highest grade available.

Development of blood extraction system [**19**]

In diabetes mellitus, in order to determine glucose levels in extracted blood, a typical diabetic patient needs to painfully extract blood between 5 and 7 times per day for glucose measurement. However, there are no painless automatic blood extraction systems for diabetic patients to enable SMBG (Self-Monitoring of Blood Glucose) [**20, 21**]. In this subsection, we propose that the medical device for Bio-MEMS, designed on the basis of a female mosquito's blood extraction mechanism, can be used to extract human blood through a painless microneedle in order to detect blood glucose levels in a diabetic patient [**19**].

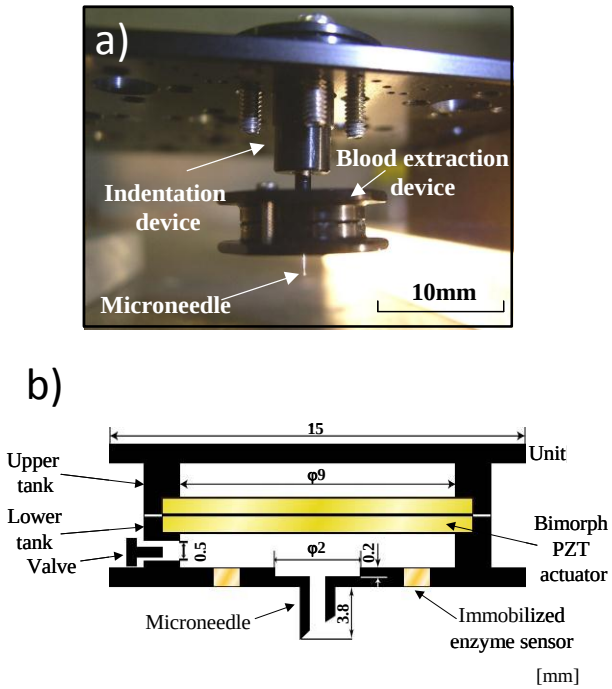

Fig. 1. (a) Schematic diagram of blood extraction device, including the microneedle, bimorph PZT actuator and immobilized biosensor. (b) Indentation and extraction device for blood sampling.

Fig. 1 shows a schematic diagram of the automatic blood extraction system. This device consists of: (1) a biocompatible titanium (a well-known biocompatible material [**22**]) microneedle indentation unit using a shape

memory alloy (SMA) [**22**] actuator; (2) a pumping unit using a bimorph PZT piezoelectric actuator; and (3) a MOSFET-type hybrid biosensor. The SMA is returned to its original shape by heating it above the Austenite phase temperature [**23**] using an electric current. It moves the pumping unit a few millimeters to generate the skin penetration force. This is held steadily for a few seconds, in order to pump and extract the blood with the negative pressure produced in the blood extraction tank using a bimorph PZT piezoelectric microactuator. The electric current is then shut off and the bias spring returns the microneedle and pumping unit to the initial position. The extracted blood is sensed by the biosensor immobilized on an Au plate embedded in the lower tank of the pumping unit, and the plate-type working electrode is connected to the gate electrode of MOSFET.

The microneedle is 3.8 mm long with an outer diameter of 100 μm to mimic the female mosquito's labium, which can extract blood almost painlessly. Based on microscopic observation, the length, inner and external diameters of the mosquito labium are 3-4 μm, 25 μm and 60 μm, respectively. Here, the amount of the α-amylase in saliva is varied by an activity change in the sympathetic nervous system, with the levels rising when the microneedle is injected into the skin, and we focused on changes in the amount of α-amylase in saliva as a pain evaluation test to decide the marginal outer diameter of the needle for the pain. To evaluate the pain associated with injection by needles with various outer diameters, two groups (data for injection needle and data for control) were statistically compared by Mann-Whitney U-assay. Significant differences were thus confirmed for all needles except those having an outer diameter of less than 100 μm. Therefore, the maximum outer diameter to mitigate the pain of injection using a microneedle is 100 μm [**24**]. It was also confirmed that the stress attributed to a pain was not dependent on the differences between the shape of the leading ends or surface condition [**23**]. However, it is difficult to produce narrow injection needles by traditional methods such as the drawing process. Here, titanium is suitable for the injection needle; however, it is known to be an unworkable material. Therefore, it is not straightforward to create microneedle of the size needed to mitigate pain. We thus proposed a novel microneedle production technique and succeeded in preparing a microneedle that mimics the female mosquito's labium [**25**]. The blood extraction device is able extract human whole blood at 2 μL/min (0.33×10^{-10} m^3/s), respectively, when a 20 V, 25 kHz AC supply is applied to the bimorph PZT piezoelectric microactuator [**19, 26**].

Therefore, the purpose of this research is to develop a high-sensitivity biosensor for automatic blood extraction and to use this sensor to detect the blood glucose in 0.5 μl of blood extracted by the automatic blood extraction system for SMBG.

MOSFET-type hybrid biosensor with extracted gate electrode

Generally, enzymes catalyze a reaction specifically with their substrates. In the case of the glucose oxidization reaction, the reaction between Go and glucose leads to a pH change and electron transfer. Studies on biosensors using an ion-sensitive field-effect transistor (ISFET), which detects pH changes, have been carried out [**27, 28**]. For example, the performance of such biosensors is based on the following reaction catalyzed by Go immobilized on the gate dielectric of the ISFET. Subsequent hydrolysis of gluconolactone to produce gluconic acid changes the pH of the solution near the biosensor surface [**29**] (**Eq. 1**).

$$\beta\text{-D glucose} + O_2 \longrightarrow \text{D-glucono-d-lactone} + H_2O_2$$
$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^- \quad (1)$$

On the other hand, research into the method for detecting electron transfer has also been carried out. The enzyme-substrate reaction leads to electron transfer; the electrode detects the generated electron. The direct electrical activation of enzymes, particularly redox enzymes, represents a general approach to stimulate the bioelectrocatalyzed oxidation (or reduction) of the substrates. The speed of electron transfer between the electrodes and the redox enzyme is fast. In the results, the electric current corresponds to the turnover rate of electron exchange between the substrate and biocatalyst [**30**]. The electron transfer by this redox reaction is generated between glucose and Go. We selected MOSFET as the transducer to detect this electron transfer.

Submicroliter volumes of blood extracted by the blood extraction device are detected using the biosensor. Extracted gate electrodes were used with the MOSFET-type biosensor system. The lower tank of the blood extraction device has the working electrodes that comprise the immobilized enzyme, the counter electrode and the reference electrode. These gate electrodes are connected via a cable with the gate electrode of the MOSFET, and the Au plate that immobilizes the enzyme is attached to the gate electrode. The lower tank has three working electrodes with more immobilized enzymes on each electrode. It is possible to detect different health index markers in the blood simultaneously by choosing an enzyme combination such as cholesterol oxidase. The diameter and purity of the Au electrode are 2 mm and 99.99%, respectively. By using this structure, the receptor and transducer of the biosensor are separated. Furthermore, it is possible to miniaturize disposable electrodes, and the miniaturization of the whole system is predicted.

The continuous electric current measurement system of the MOSFET-type hybrid biosensor with the extracted gate electrode was used. The three-electrode method makes it possible to cancel the noise by subtracting the voltage of the counter electrode from the voltage of the working electrode. In addition, the constant electric current between the source and drain of the MOSFET is fixed at 3 mA. The system can be used to measure the change in gate voltage. To confirm the ability of the MOSFET-type hybrid biosensor system to detect electrons due to the reaction between glucose and Go, three types of solution were used.

Immobilization by cross-link method

An appropriate spacer is selected to attach to the electrode. Chemical bonding between the electrode and Go is preferred because chemical bonds are stronger than physical bonds. The cross-link method is shown in **Fig. 2**. Cystamine is used as a spacer because it contains the disulfide bond that makes Au-S bonds, and it binds to the cross-link agent by an amino group. The enzyme is cross-linked by glutaraldehyde and it is immobilized onto the spacer SAMs. This method has the advantage that many enzymes can be immobilized. In this experiment, Go is immobilized on self-assembled spacers joined to Au electrode substrates by the cross-link method using BSA as an additional bonding material. The spectra of Go immobilized on an Au electrode by the cross-link method were determined by electron spectroscopy for chemical analysis (ESCA). In the ESCA spectrum, a peak was observed for amino group N 1s due to the immobilization of cystamine. Furthermore, there was confirmation of the shoulder arising from C=O in C 1s owing to the immobilization of an enzyme and carboxyl group due to the immobilization of glutaraldehyde.

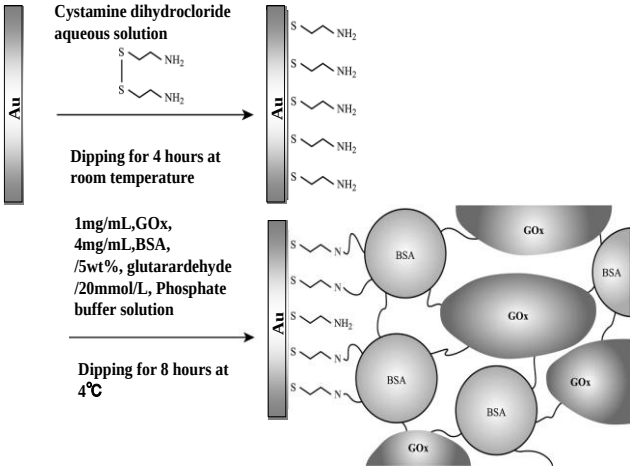

Fig. 2. Scheme of enzyme immobilization (cross-link method).

Glucose sensing in sampled blood

In this experiment, the anticoagulants heparin (0.5 mg) and sodium fluoride (100 mg) were added to 10-mL blood samples. Blood was diluted with physiological saline solution (1:50), and blood cells were filtered out. Diluted whole blood and diluted blood plasma were measured using the MOSFET-type constant electric current measurement system.

Results and discussion

Development of blood extraction system

The liquid sampling ability of the pumping unit was evaluated through a microneedle using a bimorph PZT piezoelectric microactuator. **Fig. 3** shows the extraction speed and viscosity for both water and human whole blood, where the applied voltage is 20 V. The medical device with the PZT actuator could extract water at a speed of 10 μL/min and human blood at a speed of 2 μL/min. The speed of water extraction is higher because the viscosity of human blood is four times greater than that of water. The extracted volume was sufficient to determine the glucose level in the blood, and is comparable to the volume extracted in a commercial glucose level monitor.

MOSFET-type hybrid biosensor with extracted gate electrode

Go catalyzes the oxidation reaction of glucose to produce hydrogen peroxide, and hydrogen peroxide can be oxidized on the surface of the electrode. These solutions comprised hydrogen peroxide (5 and 100 mmol/L) and ascorbic acid. **Fig. 4** shows output voltages that were detected using the MOSFET-type constant electric current measurement system for each solution. The output electric voltage could be detected using both hydrogen peroxide solutions, and the signal increased with concentration of hydrogen peroxide. Therefore, when detecting electron transfer, the electrode was able to detect electrons generated from the oxidization of hydrogen peroxide using the constant electric current measurement system of the MOSFET-type hybrid biosensor. Signals were also detected from the reaction in ascorbic acid. It is known that ascorbic acid interferes with the detection of electrons obtained by the chemical reaction between glucose and Go in electrochemistry experiments with a biosensor. Because ascorbic acid is readily oxidized, and the molecular mass of ascorbic acid is almost equal to that of glucose, a transmission membrane for filtering the ascorbic acid is necessary to prevent it from affecting the reactions between enzymes and the electrode. Thus, the sensitivity of this system is based on the higher speed addition of hydrogen peroxide and ascorbic acid.

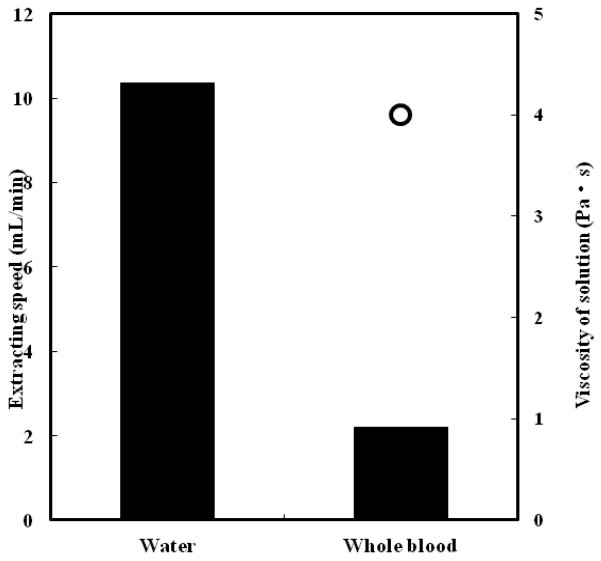

Fig. 3. Extraction speed and viscosity for water and human whole blood, where the applied voltage is 20 V. ○: Viscosity.

Glucose sensing from sampled blood

The relationship between threshold voltage and glucose concentration is shown in **Fig. 5**. Both whole blood and blood plasma showed linearity between the threshold voltage and glucose concentration. When diluted whole blood was measured, the sensitivity of this biosensor was estimated to be 61.4 V/(mol/L), and its coefficient of determination was 0.907. These values indicate that the medical biosensors are practical. Values for blood plasma were determined by the same method. The sensitivity was 171.2 V/(mol/L) and the coefficient of determination was 0.955. Good correlations were seen between threshold voltage and glucose concentration in both blood and plasma. Thus, the sensitivity was confirmed for blood using this biosensor. The results of the relationship between threshold voltage and glucose concentration was applied for calibration curves in whole blood.

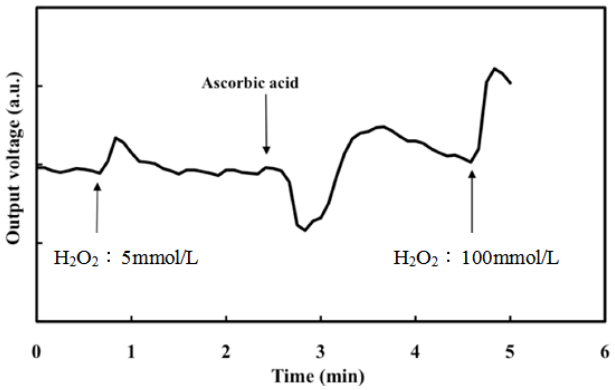

Fig. 4. Output voltages as a function of measurement time for various solutions using the MOSFET-type hybrid biosensor immobilized on an Au electrode

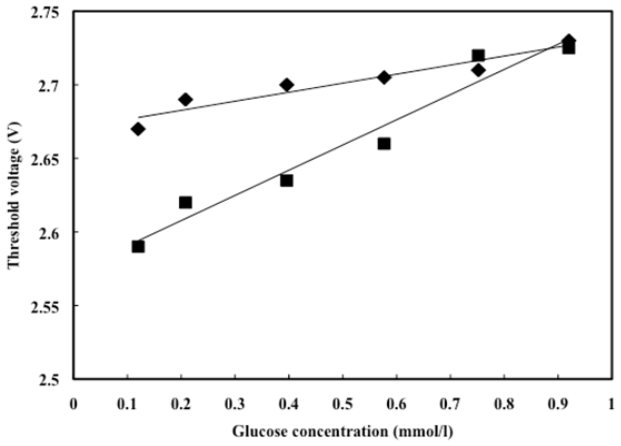

Fig. 5. Relationship between threshold voltage and glucose concentration for whole blood and blood plasma. ◆ Whole blood (y = 0.0614x+2.67); ■: Blood plasma solution (y = 0.171x+2.57).

Characterization of MOSFET-type hybrid biosensor immobilized Go

With regard to glucose concentrations (blood glucose levels) of both healthy and diabetic individuals before eating and at two hours after a meal, that of the diabetic patient is always higher than that of the healthy individual. Glucose concentrations between 3-15 mmol/L should be measured precisely using the constant electric current measurement system of the MOSFET-type hybrid biosensor. Therefore, we measured glucose concentrations of 5, 7 and 9 mmol/L. As indicated in **Fig. 1**, the blood extraction device comprises a biosensor, a microneedle, a pumping unit (piezoelectric pump) and a blood extraction tank. The microneedle and lower tank are filled with saline solution (volume, 32.5 μL).

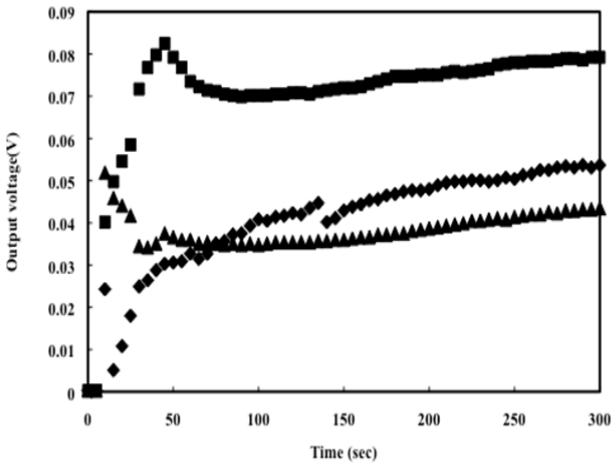

Fig. 6. Detected voltage as a function of measurement time in physiological saline solution. ◆ 0.083 mmol/L; ■: 0.116 mmol/L; ▲: 0.15 mmol/L

Blood volumes of typically a microliter are needed to detect a signal in commercial blood glucose biosensors [**15**]. When the blood extraction devices extracted 0.5 μL of blood, it was diluted 60 fold with physiological saline solution. Glucose will be diluted with saline so the glucose concentrations are in fact 0.083, 0.116 and 0.15 mmol/L. To demonstrate the same environment for glucose solutions extracted using the blood extraction device, glucose concentrations of 0.17, 0.23 and 0.30 mmol/L were prepared in beakers. These solutions were then diluted with physiological saline solution, the glucose biosensor was inserted into the beakers for 10 s, and the voltage detected using the MOSFET-type hybrid biosensor indicates the start of the measurement. Each of the various glucose concentrations was measured in turn. **Fig. 6** shows the results of the detected voltage from the biosensor, for the three glucose concentrations as a function of measurement time. The output voltage increases with the injection of glucose solution. However, the output voltage is not saturated but increases with measurement time for each concentration. **Fig. 7** shows the Au electrode (gate electrode) surface after 1 h of use. This photograph shows the laser microscopic observation of the electrode, which was attached to a polycarbonate filter with a circular hollow. It was then dipped in physiological saline solution including chlorine for 1 h. On the unfiltered part, a circular hole can be seen on the electrode. The 40 μm depth in this hole indicates that part of the filter was dissolved. The Au electrode has superior characteristics as an electrode, for example, excellent oxygen overvoltage, and it is simple to modify the electrode and spacer by

means of the Au-S bond. However, when voltages are applied between working, counter and reference electrodes in physiological saline including a chloride ion, a chloride complex is produced in the solution, leading to anode dissolution.

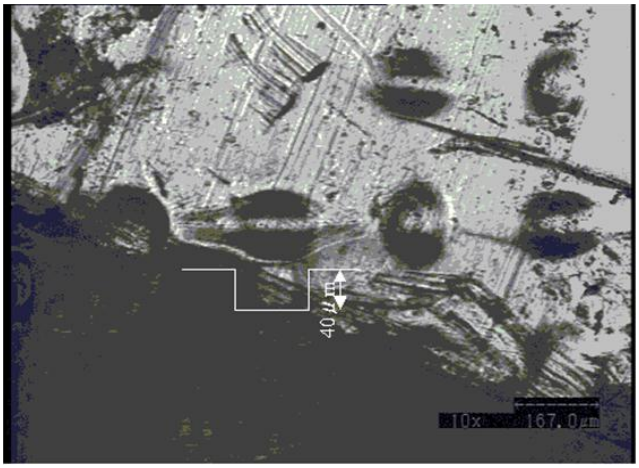

Fig. 7. Microscopic image of enzyme immobilized on Au electrode after 1 h.

In a further investigation, **Fig. 8** shows the output voltage for three values of glucose concentration solution as a function of measurement time, where pure water (Milli-Q) was used as the diluted solution for comparison with the changes in output voltage for physiological saline solution. Glucose concentration was dependent on output voltage; thus, high glucose concentrations have a good relationship with output voltage. The voltage stabilized at higher glucose concentrations (> 0.12 mM), and later, the output voltage tended to saturate for about 120 s. In addition, the averaged output voltage after 120 s using Milli-Q water as the dilution agent increased linearly with glucose concentration. These glucose concentrations were measured up to human body glucose standard levels.

Output voltage for glucose solutions collected using blood extraction device

The output voltage was measured for the glucose solution dissolved in Milli-Q water collected using the bimorph-type PZT piezoelectric extraction device, including the biosensor, microneedle, actuator in the blood tank and pumping system. Sampling time was 5 s using the pumping system. The conditions for the bimorph-type PZT actuator were 20 V and 25 kHz AC. The pumping time was 30 s. The blood extraction device collected a few microliters of 0.5 mmol/L glucose solution. **Fig. 9** shows the output voltage as a function of measurement time using the glucose biosensor. The output voltage increased when the glucose solution was extracted for 30 s, and showed a peak voltage. Subsequently, the output voltage became saturated and stabilized, and glucose concentration measurement was started about 150 s later. Reproducible results were obtained using this system. Thus, glucose concentration was dependent on output voltage, and the blood extraction device could use one tank to simultaneously extract and detect the signal. This suggests that the detection of various substrates is possible by changing the enzyme that was immobilized on the extracted gate electrode of the biosensor. However, we still need to calibrate the output voltage in terms of blood glucose levels.

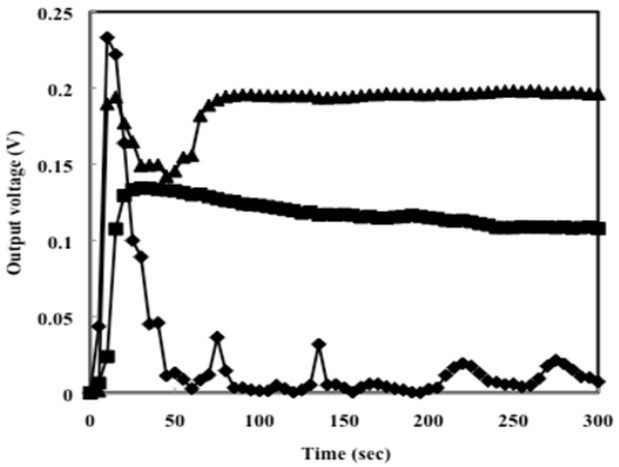

Fig. 8. Indicated voltage as a function of time for various glucose concentrations. ◆ 0.083 mmol/L; ■: 0.116 mmol/L; ▲: 0.15 mmol/L.

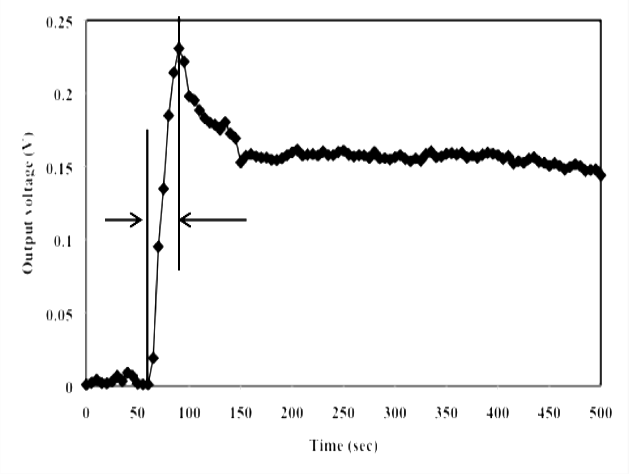

Fig. 9. Indicated voltage as a function of time. ◆ 0.083 mmol/L.

Conclusion

We developed a MOSFET-type hybrid biosensor-immobilized Go on an Au-extracted gate of MOSFET by the cross-link method. The performance of the produced biosensor was evaluated, and the following information was obtained.

a) Whole blood showed a lower output voltage than blood plasma. Consequently, it would be sensible to use the detector for blood plasma rather than whole blood.
b) The output voltage for Milli-Q water as the dilution agent increased linearly with increasing glucose concentration.
c) Extracted solutions of around a microliter were sufficient to produce a signal that the biosensor was able to detect.

These experiments showed that the performance of the

MOSFET-type hybrid biosensor was adequate as the sensing unit in HMS.

Acknowledgements

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