Transactions of the Materials Research Society of Japan 30[4] 1247-1250 (2005)

Design of Micro-biosensors in Healthcare Chip for Convenient Blood Diagnostics

Madoka Takai[1], Hiroki Ogawa[2], Masao Nagai[2], Kazuhiko Ishihara[1] and Yasuhiro Horiike[3]

[1]Department of Materials Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-8656, Japan

[2]Adbic Inc., 2-1-6 Sengen, Tsukuba, 305-0047, Japan

[3]Biomaterials Center, National Institute of Materials Science, 1-1 Namiki, Tsukuba 305-0044, Japan

Fax : +81-3-5841-8647, e-mail : takai@bmw.t.u-tokyo.ac.jp

ABSTRACT

To check our daily health condition, a disposable and cheap healthcare chip allowing multi-item diagnostics such as pH, K^+, Na^+, Blood Urea Nitrogen (BUN) and Glucose, in trace of blood has been developed using plastic plate. Stability in self-potential of each micro-sensor fabricated on printed carbon electrode was strongly influenced on the size of carbon particles and its binder. High ion selectivity of Na^+ and K^+ micro-ion-sensors used polymer membrane including ionophore measuring by potentiometric method were obtained by controlling mole ratio of anion-exclusion agents to inophores. Sensitivity of BUN sensor immobilized urease on a hydrogen sensor made of non-conductive polypyrrole became better by immobilization method of enzyme using poly-ioncomplex membrane. The significant design of micro-glucose sensor measuring by amperometric method is highly uniform immobilization of electron mediator and preventing plasma protein adsorption in blood. The uniform fabrication on carbon electrode was achieved by using the polymer complex contained mediator, and biocompatible phospholipid polymer coating inhibited non-specific protein adsorption. The design of channels with a blanched capillary was effective for preventing mutual interference due to the enzyme-reaction. The electrochemical blood diagnostics chip employing screen-printed carbon electrodes on a polymer film allowed to achieve a cheap, multi-item and simultaneous measurement.

Key words: micro-biosensor, disposable healthcare chip, blood diagnostics, electrochemical measurement, biocompatibility

1. INTRODUCTION

An analysis of blood component to check our daily health condition is quite important to prevention of illness. For that purpose, simple, convenient, cheap, and small healthcare analyzer combined with painless needle for blood collection should be required. Previously, we developed the healthcare chip consisting of a painless needle, an U-shape channel for the on-chip centrifugal separation of a blood, ion sensitive field effective transistor (ISFET) sensors, and a low voltage-driven electro-osmosis flow (EOF) pump made by quartz was developed on a polyethylene terephthalate (PET) plate [1]. Since the chip should be cheap and disposable as a home medical device, a new healthcare chip integrating several bio-sensors, such as blood urea nitrogen(BUN) and glucose sensors, in addition to ion-sensors fabricated on screen printed carbon electrodes instead of ISFET has been developed. The photograph of healthcare chip with multi-item electrochemical sensor are shown in Fig. 1, where the blood was introduced by a pump set at outside[2,3], because the costs of ISFET sensor and EOF pump are quite expensive comparing with other materials. In this paper, we report suitable design in membrane structures of ion sensors for pH, Na^+, and K^+ and enzyme-immobilized sensors for BUN, and glucose on the carbon electrodes for improvement of the sensitivity, stability, and reliability. Especially, it is considered that a high accuracy measurement of blood glucose concentration in micro sample strongly depends on plasma protein adsorption on sensor surface, and limitation of dissolved oxygen in plasma. Therefore, we also report the high performance micro-glucose sensor using electron mediator and biocompatible phospolipid polymer[4]. As well as sensor membrane, we showed channel design suited to multi-item measurements for preventing of mutual interference among sensors. Furthermore we introduced total operation

method using this healthcare chip for analysis in a small blood volume around 1μL.

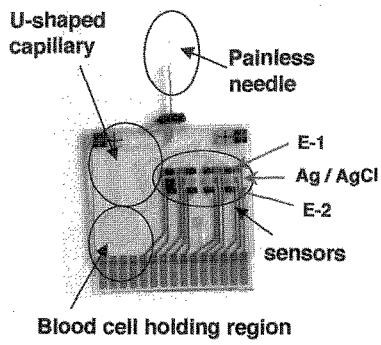

Fig.1 Photograph of disposable healthcare chip.

2. MATERIALS and METHODS

The chip shown in Fig. 1 was fabricated as following step. First, AgC contact electrode was formed on a polyester sheet by screen-printed method, next carbon as a working electrode, and Ag/AgCl as a reference electrode were also formed by screen-printing on the AgC. Second, insulating epoxy film was bonded on the sheet by heating. After opening of electrode window by photo-lithography, sensor membranes for measuring Na^+, K^+, pH, blood urea nitrogen (BUN) and glucose were dispensed or electro-polymerized on an open area around $0.1mm^2$ of carbon electrode. Membrane structures on ion- and bio- sensors were summarized in Table 1. Non-conductive polypyrrole(PPy) was prepared by electro-polymerization according to the reference[5] as pH sensor. Bis(benzo-15-crown-5), and Bis(12-crown-4) purchased from Fulka.Co. were used as a ionophore for K^+, and Na^+ sensors. Urease(1500unit/mL) for BUN sensor was immobilized by Polyioncomplex(PIC) membrane using Poly-L-lysine hydrobromide(PLL) and PPy. Ferrocene entrapped with vinyl polymer was used as an electron mediator and glucose oxidase (1500unit/mL) was immobilized by PIC membrane. Poly(solium 4-stylen-sulfonate)(PSS) and PLL were used as anionic and cationic polymers, respectively. Finally, phospholipid polymer: poly(MPC-*co*-n-butyl methacrylate)(PMB30) (0.3wt% EtOH solution) was coated on the PIC layer. Finally, a PET plate embossing with a micro-channel patterns was bonded with the polyester sheet via the photo-polymerized epoxy film. A pocket located at a middle position of the U-shape channel plays a part of collection of red-blood cells after an on-chip centrifugation. Before installing a painless needle, the inner wall of channel was coated by the PMB to suppress absorption of proteins in plasmas.

Table 1 Film structures of pH, Na^+, K^+, BUN and glucose sensors.

pH	Non-conductive PolyPyrrole(PPy)
Na^+	Na^+ ionophore, plasticizer, anion-exclusion-agent and Poly(Vinyl Chloride) :PVC
K^+	K^+ ionophore, plasticizer, anion-exclusion-agent and PVC
BUN	PMB30 / Urease / non-conductive PPy
glucose	PMB30 / Glucose-oxidase / ferrocence

3. RESULTS AND DISCUSSION

3-1 Reliability of carbon electrode

Prior to fabricating ion sensor membranes, characteristics of the screen-printed carbon electrode were investigated to obtain stability and reliability of ion sensors. Many attentions were paid for uniform dispersion of carbon particles with grain size less than 10 μm and a selection of appropriate insoluble binder for solvents of ionospheres to achieve highly precise measurements. Figure 2 shows the results of reliability of carbon electrodes by an evaluation of self-potential in 1wt% of H_2SO_4 solution. Rest-potentials of 10 electrodes in type A show very widely, while those in type B electrodes are stable value around 300mV to 350mV. From cross sectional SEM image of carbon electrode as in Fig. 2, electrode of type A has large grains comparing with it of type B, and it makes disordered interface between AgC and carbon. Therefore, uniform interface is thought to be important to reliability of carbon electrode by screen-printing.

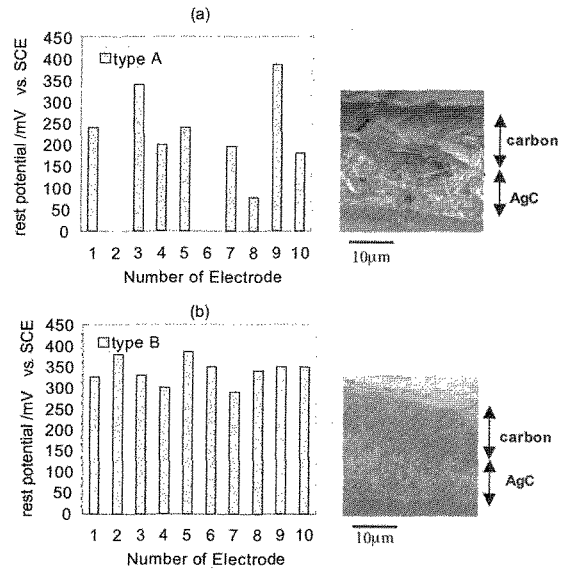

Fig.2 Evaluation of stability in 10 carbon electrodes, and cross sectional SEM images of each typical carbon electrode. Type A electrode consists of large graphite particle, type B electrode consist of small grains.

3-2 High selectivity on ion sensors

As for the blood analysis, high ion sensing selectivity is required for Na^+ and K^+ ions, because concentration of sodium ions is about 30 times higher than that of potassium ion in human plasmas. Control of the mole ratio of anion exclusion-agent, triskis(4-chorophenyl)borate potassium salt (k-TCPB), to ionophore and their immobilizations on the carbon electrodes allowed us to provide high ion selectivity of $Na^+/K^+=10^2$ and $K^+/Na^+=10^3$. As shown in Fig. 3, representative effect on the mole ratio of anion exclusion-agent to ionophore for K^+ ion sensor is depicted. Large amount of anion-exclusion agent in membrane pulled any cation due to negative charge of the anion-exclusion agent. Under the condition of 1:0.5(ionophore:k-TCPB), K^+ sensor has an sufficient properties for blood analysis.

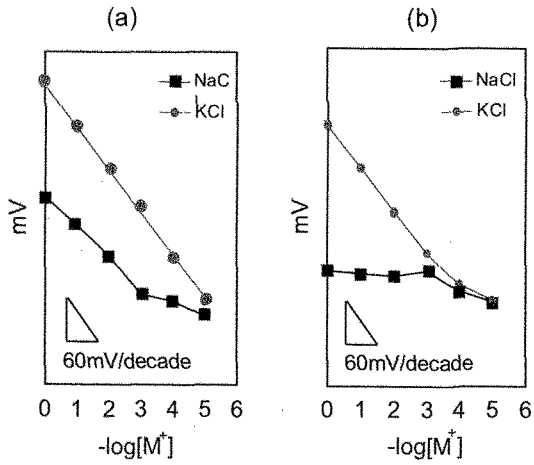

Fig. 3 Calibration curves for K^+ sensors; (a)ionophore:anion-exclusion-agent=1:1, (b)ionophore :anion-exclusion-agent=1:0.5, respectively.

3-3 Design of micro-glucose sensor

For amperometric micro-glucose sensor, high reliability and sensitivity were achieved by the design of electron mediator as well as immobilization of enzyme. Firstly, we investigated the relationship between uniformity in mediator on the carbon electrode and sensor performance. The sensor fabricated using the condition of large ferrocene particles as in Fig.4(a) had poor reliability and wide fluctuation among sensors. While, the sensor using the condition of highly uniform mediator as in Fig.4(b) showed high reliability for glucose measurement. Another concern obtaining high reliability for amperometric sensor is control the degradation by protein adsorption on sensor surface. Biocompatible polymer, PMB, was

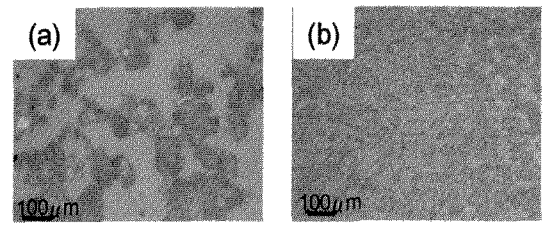

Fig. 4 Optical microscope images of (a) ferrocene dissolved in distilled water, (b)ferrocene and polyvinyl butyral dissolved in dichloromethane

used for suppression of protein adsorption. Figure 5 shows a representative effect of PMB coating on micro glucose sensor. Current was measured at applied voltage of +350mV vs. Ag/AgCl using D-glucose solution (10mmol dm^{-3}) in phosphate-buffer saline (PBS) and human serum (Glucose: 5.6mmol dm^{-3}). First, we measured the glucose concentration of 10mmol dm^{-3} in PBS. The current densities of all sensors (two sensors were coated with PMB, and the others were coated without PMB) show almost the same value. Then glucose concentration in human serum was measured using the four electrodes. The current density of the sensors with PMB coating is greater than that of the sensors without PMB coating. In contrast, the current densities of the sensors with/without PMB coating increased linearly with increasing glucose concentration from 0 to 10mmol dm^{-3} in PBS. These results showed that the drastically decrease of the current density in sensors without PMB coating was caused by adsorption of proteins in human serum. Therefore, PMB coating on micro-electrochemical glucose sensor is necessary for blood analysis to obtain high reliability.

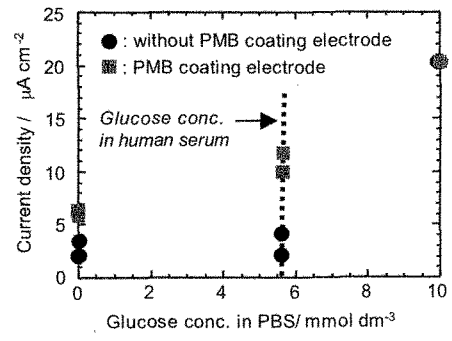

Fig.5 Effect of biocompatible treatment on micro-glucose sensor using PMB.

3-4 Control of mutual interference

ISFET used in the previous healthcare chip[1] were arranged one single capillary, whereas concentration of Na^+ and K^+ ions were measured by the potentiometry using their inherent ionophores. In the present multi-items diagnostics, however, a mutual interference was

likely to occur among sensors via by-products generating from the enzyme reaction on the sensor electrodes. Hence we investigated effects of the mutual interference using newly design healthcare chip developed here, which had four branch capillaries. When BUN and pH sensors were set in the same channel as shown in Fig. 6(a), since an urea was hydrolyzed by an urease under the reaction of $NH_2CONH_2 + 2H_2O + H^+ \rightarrow 2NH_4^+ + HCO_3^-$, the pH on pH sensor increased with the rise of urea concentration. In Fig 6, arrows illustrate flow directions. Even if the flow direction was reversed, a range of pH change on pH sensor was reduced to only 0.2. Eventually, dividing electrodes for BUN and pH sensors in different channel successfully controlled a mutual interference as is seen in Fig.6(b).

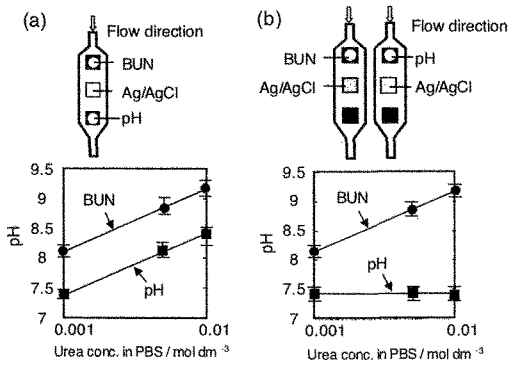

Fig.6 Controlling of mutual interference using branch channel. (a) BUN and pH sensors are set in the single channel, (b) BUN and pH sensors are set in the branch channel.

3-5 Blood diagnostics

A blood diagnostics was carried out as follows: At first calibration curves were obtained using a standard solution, and then the whole blood was collected from the painless needle using a pump set outside. Blood cells and plasmas were separated by the on-chip centrifugal separation. Finally plasmas were extracted and introduced into channels arranging on electrodes, while blood cells were remained in the pocket of the U-shape channel. Figure 7 shows representative example of calibration curves for BUN sensors, where analytical values of the concentration in human plasmas are also listed. Calibration point on chip means the measurement data after on chip centrifugal separation using 0.001mol dm^{-3} of urea dissolved in PBS. Shadow area indicated the region of normal BUN value on healthy people. Here, it was confirmed that the slope of calibration curve does not change after centrifugal separation. BUN concentration in human plasma was successfully measured trough the calibration process. Not only BUN

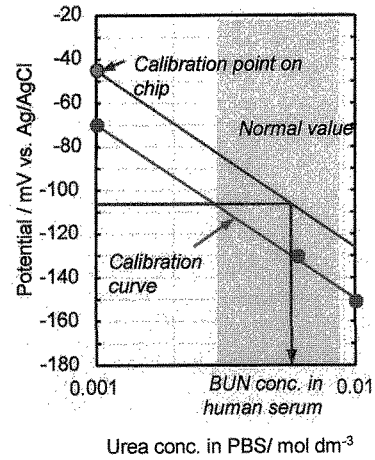

Fig.7 Calibration curves of BUN sensor for practical use.

diagnostics in human plasma, but also pH, Na^+, K^+, and glucose concentrations were measured using same calibration process, though those data were not shown. Therefore, the reliability of sensors is considered to be satisfactory for the medical use. Total analytical time spent from the calibration to obtaining analytical results was limited to almost 3min.

4. CONCLUSION

Disposable healthcare chip enabling to check multi-makers in a trace of blood has been developed based on investigation of electrochemical ion, and biosensors on printed carbon electrodes. A carbon grain size less than 10μm and appropriate binder of electrodes led to stable and reliable on sensor characteristics. High ion selectivity on Na^+, and K^+ ion sensors was obtained by controlling the mole ratio of anion exclusion-agents to ionophores. Reliability of glucose sensor was improved by uniform immobilization of mediator. PMB coating on amperometic glucose sensor has a grate effect to suppress absorption of proteins in plasma, and the branch channel in healthcare chip has key design rule for preventing mutual interference due to the bio-reaction. The healthcare chip having electrochemical detection has an advantage for convenient blood analysis.

5. REFERENCES

[1] A. Oki, *et al,* Jpn. J. Appl. Phys. Vol.42, Part 1 , No.6A , 3722-3727 (2003).

[2] H.Ogawa, *et al, Proceedings of micro-TAS* **Vol.1**, p.741-743(2003)

[3] M. Takai, *et al, Proceedings of micro-TAS* **Vol.1**, p403-405 (2003)

[4] K. Ishihara, *et al,* J. Biomedical Materials Research. Vol.26, 1543-1552 (1992).

[5] T. Osaka, *et al J. Electroanal. Chem.*, **372**, 201(1994).

(Received December 24, 2004; Accepted June 30, 2005)