유체소자가 집적화된 면역검사용 휴대용 CMOS 바이오칩의 분석

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ANALYSIS OF FLUIDIC BEAD CUBE EMBEDDED PORTABLE CMOS SENSING SYSTEM FOR IMMUNO REACTION MONITORING

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Abstract

This paper describes the novel immunoassay sensing system for a portable clinical diagnosis system. It consists of a bead cage reactor and a CMOS integrated biosensor. It showed the simple and easy antibody coating method on beads by flow-through avidin biotin complex technology in a microfluidic device. It showed just 90 nL sample consumption and good result for the application of alpha feto protein. The bead cage reactor has the role of the antibody coating, antigen binding and enzyme linking for the electrochemical sensing method. The CMOS biosensor consists of ISFET (ion selective field effect transistor) biosensor and temperature sensor for detecting pH that is the byproduct of enzyme reaction. The sensitivity is 8 kHz/℃ in a temperature sensor and 33 mV/pH in a pH sensor. After filling the 15 um polystyrene beads in bead cage, antibody flowed and reacted to beads. Subsequently, the biotinylated antigen flowed and bound to the antibody and GOD (glucose oxidase)-avidin conjugate flowed and reacted to the biotin of the biotinylated antigen. After this reaction process, glucose solution flowed and reacted to the GOD on beads. The hydrogen was generated by glucose-GOD reaction. And it was detected by the pH sensor.

I. Introduction

Our system consists of a bead cage reactor and a CMOS integrated biosensor as shown in Fig. 1. The bead cage reactor was presented at the other conference [1]. The results of the bead cage reactor are shown in Fig. 2. The bead cage reactor showed a simple and easy antibody coating method applied to beads by flow-through avidin biotin complex technology in a microfluidic device with a minimized sample consumption. However, it needed confocal microscope which disturbed full portable microTAS. Therefore, we developed a CMOS integrated biosensor and tried to integrate the biosensor and the bead cage reactor. The bead cage reactor facilitates the antibody coating, antigen binding and enzyme linking in the application of the electrochemical sensing method [2]. The CMOS biosensor consists of ISFET (ion selective field effect transistor) biosensor and temperature sensor for detecting pH which is the byproduct of enzyme reaction. The main focus

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here is that enzyme linked immunoassay is possible with pH ISFET in nanoliter micro reactor. Even though the generated protons which are the product of ELISA reaction have small quantities in the application of low diluted antigen detection, they can affect the pH level enough in that nanoliter volume of reactor.

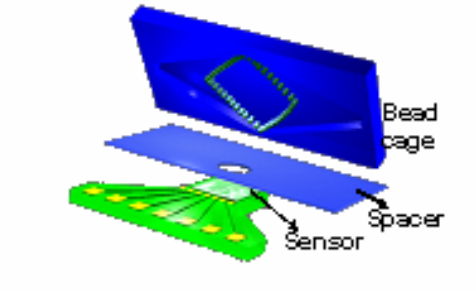

Figure 1. System schematics

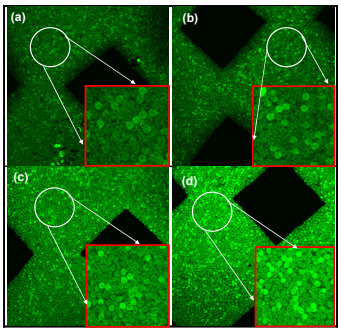

Figure 2. Confocal microscope images for different AFP concentration (a) 284 ng/ml (b) 568 ng/ml (c) 1.4 ug/ml (d) 2.8 ug/ml

II. . DESIGN AND CMOS FABRICATION

2.1 Temperature sensors

In this study, six lateral BJTs were connected serially to maximize the temperature sensitivity. The change of base-emitter junction voltage as temperature is multiplied by the six number of serial BJTs. The temperature output was converted to the frequency output to differentiate output signal information of temperature and pH by the VCO (voltage controlled oscillator). The result is shown in Fig. 3.

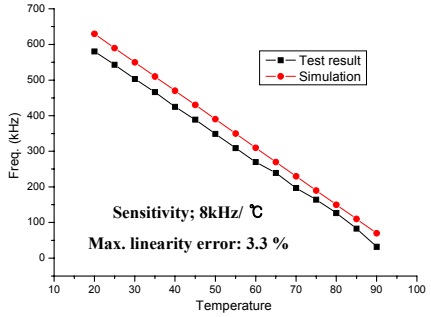

Figure 3. Test results of the temp. sensor

2.2 ISFET (Ion selective field effect transistor) system

The same or even better results can be obtained by using two ISFETs with different pH-sensitivity, such as a SiN gate ISFET in combination with a SiO gate ISFET. [3] There is a difference potential between the SiN gate ISFET and MOSFET that has the polysilicon gate with same gate geometry of SiN gate ISFET. That result is differentially amplified again with the result of the SiO gate ISFET (REFET). In this process, the ISFET gate is biased by the Pt pseudo reference electrode and the noise signal in pseudo electrode is removed by the common input characteristics of differential amplifier. Fig. 4 shows the final test result of pH sensor system. Hystresis phenomenon occurs during this test on the pH. The maximum pH error between two pH tests was found to be about 10 mV, i.e., a difference of about 0.21 pH. However, immuno test used the pH decreasing effect detecting the proton generation between enzyme and enzyme oxidase.

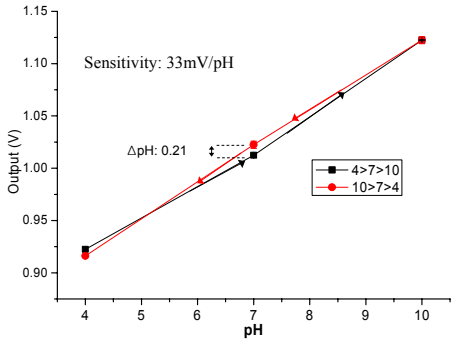

Figure 4. Test results of ISFET

2.3 CMOS fabrication

N-well, 1-poly and 1-metal CMOS process of ISRC (inter-university semiconductor research center) in SNU was adopted and modified at the back-end process for ISFET fabrication. The sensing dielectrics was deposited between ILD (inter-layer dielectric) and IMD (inter-metal dielectric) layer.

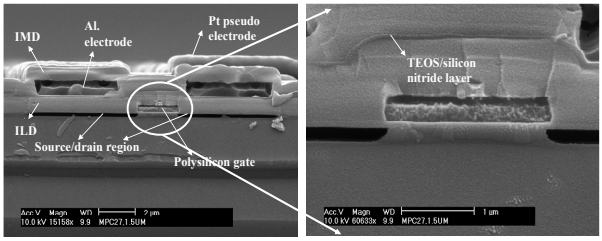

Figure 5. Cross-section of the fabricated sensors

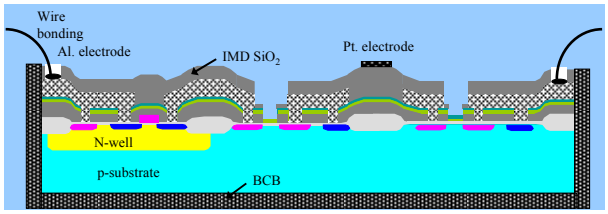

Figure 6. Packaging concept of the sensors

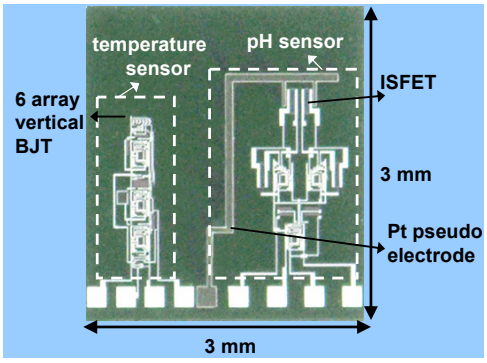

Figure 7. The fabricated CMOS sensors

III. INTEGRATION

For the thin space layer between the bead cage and CMOS biosensor, the anti-adhesion layer (tridecafluoro-1, tetrahydrooctyl-1-tricholorosilane) was spin-coated to the silicon wafer. This prevents the thin PDMS layer from tearing when the space layer is peeled off. Therefore, PDMS was spin-coated by the thickness of 20 um after the anti-adhesion layer coating and baked on a 75 ℃ hot plate. A thin PDMS sheet was punched through it with 1 mm diameter in which the reacted enzyme product can react with the sensing surface of the ISFET, and then, diced and peeled off. First, the bead cage PDMS sheet and space layer was bonded irreversibly after O2 plasma treatment. Then, the bead cage that was bonded with the space layer was bonded to the PDMS around the CMOS biosensor again without performing the plasma treatment as shown in Fig. 8. By doing so, CMOS biosensor can be reused by washing it after the test. After the antigen diagnosis test was performed with beads in the bead cage, the bead cage part was separated from the CMOS biosensor. The PDMS fluidic part was used as a disposable device and CMOS biosensor was used as a reusable device.

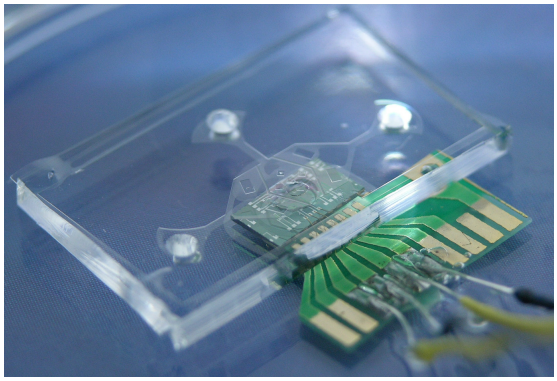

Figure 8. The integration result

IV. RESULTS AND DISCUSSION

After filling the 15 um polystyrene beads in bead cage, the antibody flowed and reacted with the beads. Subsequently, the biotinylated antigen flowed and bound to the antibody while GOD (glucose oxidase)-avidin conjugate flowed and reacted with the biotin of the biotinylated antigen. The antibody, anti-AFP, is immobilized to the bead surface by avidin-biotin conjugation (ABC) methodology. Then, the antigen is conjugated with GOD by ABC methodology. The antigen, alpha feto protein (AFP), is biotinylated in NHS-biotin and reacted with avidin-GOD conjugate. After this reaction process, glucose solution flowed and reacted with the GOD on beads. Hydrogen was generated by glucose-GOD reaction. As a result, the hydrogen peroxide generates two proton, two electrons and one oxygen molecule. This was detected by the ISFET. The

reaction sequence is shown in Fig. 9 and the test result of alpha feto protein is in Fig. 10. The glucose changes to the gluconic acid and the glucose oxidase remains the same due to its catalyst property. The conventional amperometric sensor detects the electron concentration by measuring the current, while pH ISFET detects the proton concentration by measuring the pH.

The beads have a 3-dimensional structure where the antigen-antibody binding normally occurs at the surface of the sphere. This means the generated proton changes the whole pH in bead cage and not just around the surface. This in turn generates stability and further changes to the pH compared with the surface 2-D conventional reaction. In addition, the pH saturates more rapidly in the case of a minute glucose reaction because the reaction takes place in the nanoliter bead cage volume. This shows that the platform can be used to initiate the ng/ml immunosorbant assay.

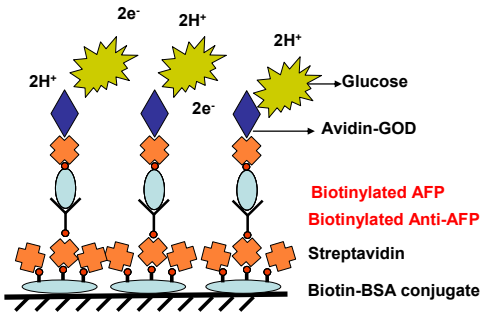

Figure 9. Coating schematics for immunoassay

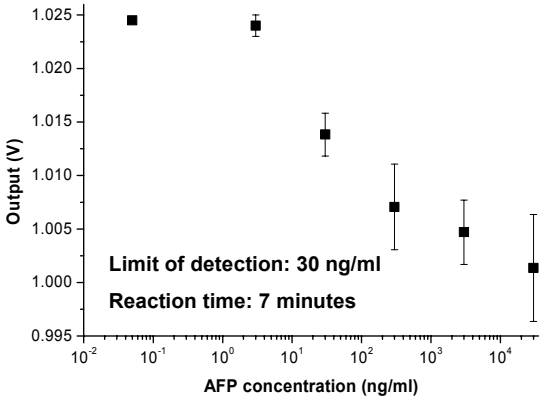

Figure 10. AFP diagnosis test

Table 1. The sequential coating recipe

sequence	condition	reaction time
bead filling	15 um polystyrene beads in 10X PBS	20 seconds
BSA coating	1.2 mg/ml biotin-BSA in 10X PBS	5 minute
washing step	0.15 wt% Tween 20 in 10X PBS	1 minute
steptavidin coating	0.5 mg/ml streptavidin in 10X PBS	5 minute
washing step	0.15 wt% Tween 20 in 10X PBS	1 minute
antibody coating	1.5 mg/ml anti-AFP in 10X PBS	5 minute
washing step	0.15 wt% Tween 20 in 10X PBS	1 minute
antigen coating	biotinylated anti-AFP in 10X PBS	10 minute
washing step	0.15 wt% Tween 20 in 10X PBS	1 minute
GOD coating	1.4 mg/ml avidin-GOD in 10X PBS	10 minute
washing step	0.15 wt% Tween 20 in 10X PBS	1 minute
glucose reaction	2.5 mM PBS, 150 mM NaCl, 25 mM glucose	

V. CONCLUSIONS

In this work, a new type of immunoassay system that can be used as a portable diagnosis system by utilizing an integrated signal processing circuit was proposed. It took about 20 minutes to produce a full recipe of protein patterning. We successfully diagnosed the AFP from 10^{-8} to 10^{-6} g/ml which is over the desired range of AFP concentration in clinics in general.

REFERENCES

[1] Y. W. Jeong et al, Transducers'05, Seoul.Korea, pp.1155-1158, Jun.2005

[2] E. J. Kim et al, Sensors and Actuators B, 79, 87, (2001)

[3] P. Bergveld and A. Sibbald, Analytical and biomedical applica-tions of ion-selective field-effect transistors, Wilson and Wilson's comprehensive analytical chemistry 23, Elsevier science publisher, (1988)