CAPILLARY SENSOR ARRAY CHIP AS A "SAMPLE-TO-ANSWER" DEVICE FOR SIMPLE, RAPID, AND MULTIPLE COMPONENT ANALYSIS OF SERUM SAMPLE

Yusuke Kimura, Terence G. Henares, Shun-ichi Funano, Tatsuro Endo and Hideaki Hisamoto
Osaka Prefecture University, JAPAN

ABSTRACT

This paper reports a novel microdevice showcasing the rapid and simultaneous multi-analyte sensing of different serum components; glucose, cholesterol, pH and alkaline phosphatase (ALP). Discrimination between normal and high analyte concentration in a serum sample was achieved and results were obtained within minutes after sample introduction. The complexity of microdevice fabrication and fluid handling from our previous system, capillary-assembled microchip (CAs-CHIP) [1-2], has been circumvented in this work that led to a simple fabrication and easy operation. Furthermore, we have exploited the fluorescein-based probes for simultaneous multi-analyte sensing by using only one fluorescence filter.

KEYWORDS

Serum analysis, glass capillary sensor, multi-analyte sensing

INTRODUCTION

Blood serum contains essential biomaterials that are associated with disease progression. Monitoring of such proteins or metabolites present in serum sample is of utmost importance for disease prevention or treatment. However, the current technology for serum analysis is time consuming and utilizes relatively large amount of expensive reagents Therefore, the development of a simple, rapid and reliable sample-to-answer bioanalytical microsystem with the ability to analyze multiple components in a given biological sample is vital in the field of micro-total analysis systems. Such research thrust is highly regarded for clinical and medical applications.

EXPERIMENT

The general concept of the capillary sensor array and operation is depicted in Figure 1. The device is divided into two parts, the capillary embedding black PDMS part [3] and the PDMS reservoir, both of which were prepared by simple molding. The square capillary sensors (300μm outer dimension) were embedded into the black PDMS square microchannels (300μm), and then combined with the PDMS reservoir to realize the multi-capillary sensor array chip. The capillaries for glucose, cholesterol, pH and ALP sensing were prepared by immobilizing the molecular probes associated for each analytes with polyethylene glycol acting as scaffold inside the square glass capillary (100μm inner dimension). The spiked and unspiked serum samples were introduced into the capillary via capillary action and allowed to react with specific molecular probe present in the capillary. Sample evaporation was prevented by dropping PDMS oil on both ends of the capillary array sensor chip. Fluorescence response was measured using a fluorescence microscope and processed with ImageJ software.

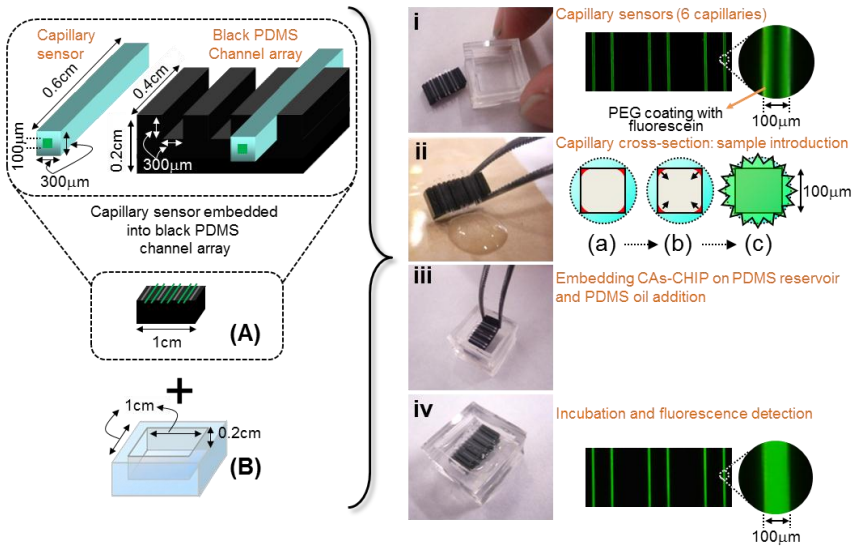

Figure 1: Basic concept and simple operation of the multi-capillary sensor array chip. Major parts of the chip (A) capillary embedding black PDMS, (B) PDMS reservoir and photos of the operation steps: i- parts of the chip with six capillaries embedded into the black PDMS; ii-stages of sample introduction: (a) capillary sensor with reagent immobilized on the four corners of the capillary, (b) reagent release by capillary action and (c) reagent-analyte reaction generating fluorescence; iii-assembly; iv-sealed with PDMS oil droplet and fluorescence detection.

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

RESULTS AND DISCUSSION

The current device is an open-type and the size of the capillary embedding black PDMS is shorter than the length of the capillary sensor. This approach permits a reproducible device fabrication and facile fluid handling. The capillary sensors take advantage of a molecular probe that yields green fluorescence response whether the reaction is enzymatic or protolytic. Figure 2 depicts the chemical reactions inside the sensing capillary. Such capillary sensor design permitted single fluorescence filter utilization, which led to simultaneous biosensing. In addition, more sensitive fluorescence measurement is obtained using the black PDMS. The bioanalytical features of various capillary biosensors are displayed in Table 1. All of the analytes registered an analysis time within 10 minutes and a coefficient of variation of not more than 9%. These results proved a rapid and reliable measurement of glucose,

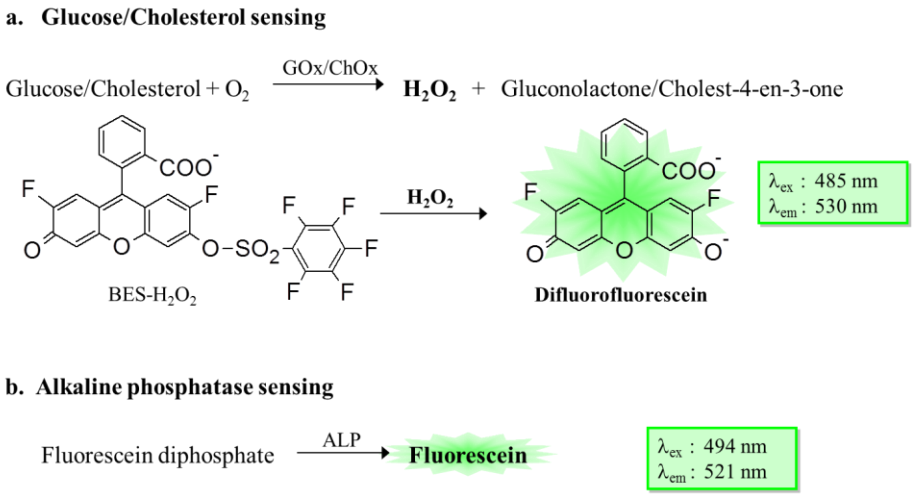

Figure 2: B Enzymatic reactions in various sensing capillaries containing different enzymes like glucose oxidase (GOx), cholesterol oxidase (ChOx) and alkaline phosphatase(ALP).

Table 1. Basic bionalytical characteristics of the capillary sensors

Capillary sensors	Analysis Time [min]	Response Range (Serum Level)	RSD (n=3)
Glucose	5	0.1 – 1.5 (0.7-1.1) [mg/mL]	7.7%
Cholesterol	8	0.5 – 2.5 (1.4 – 2.2) [mg/mL]	5.3%
ALP	10	0.05 – 1.0 (0.1 - 0.3) [unit/mL]	8.8%
pH	~ 1	pH: 7.1–7.6 (pH: 7.3±0.05)	5.5%

cholesterol, pH, and ALP. Moreover, the normal level of each analytes in the serum sample is within the response range of the capillary sensor, which implies that accurate measurement is expected. Figure 3 demonstrates the ability of the capillary sensor array chip to discriminate normal and high level of analytes in a serum sample. An increase in fluorescence response is observed when a particular analyte is spiked on a normal serum sample while other capillary sensors did not respond.

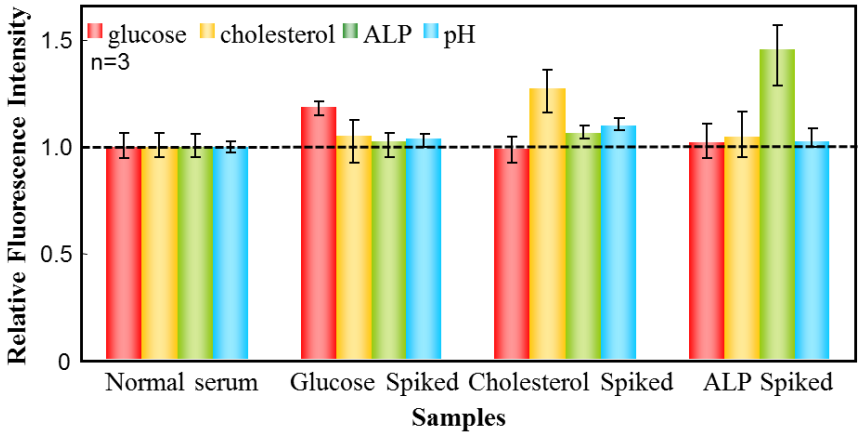

Figure 3: Comparison of the spiked and unspiked normal serum sample using the multi-capillary sensor chip.

CONCLUSIONS

A simple microdevice fabrication and an easy assay operation for the rapid, simultaneous multiplexed measurement of glucose, cholesterol, ALP and pH in serum sample was successfully demonstrated. The integration of various sensing conduits into the PDMS channel array that yields the same green fluorescence response upon reaction with an analyte permitted single fluorescence filter utilization. Thus, simultaneous multi-component measurement of serum sample was realized. This open-type CAs-CHIP was able to discriminate between the normal and high serum level of various analytes with purely single-step operation and within minutes upon sample introduction. The proposed bioanalytical tool could be applied for disease prognosis or diagnosis.

REFERENCES

[1] H. Hisamoto, Y. Nakashima, C. Kitamura, S.-i. Funano, M. Yasuoka, K. Morishima, Y. Kikutani, T. Kitamori and S. Terabe, *Capillary-Assembled Microchip for Universal Integration of Various Chemical Functions onto a Single Microfluidic Device*, Anal. Chem., 76, 3222-3228, (2004).

[2] T. G. Henares, M. Takaishi, N. Yoshida, S. Terabe, F. Mizutani, R. Sekizawa and H. Hisamoto, *Integration of Multi-Analyte Sensing Functions on a Capillary-Assembled Microchip: Simultaneous Determination of Ion Concentration and Enzymatic Activities by "Drop-and-Sip" Technique*, Anal. Chem., 79, 908-915 (2007).

[3] Y. Fujii, T. G. Henares, K. Kawamura, T. Endo, H. Hisamoto, *Bulk- and Surface-Modified Combinable PDMS Capillary Sensor Array as Easy-to-Use Sensing Device with Enhanced Sensitivity to Elevated Concentrations of Multiple Serum Sample Components*, Lab Chip, 12, 1522-1526 (2012).

CONTACT

Hideaki Hisamoito Fax:+81-72-254-9284; Tel:+81-72-254-9285 or hisamoto@chem.osakafu-u.ac.jp