AN INTEGRATED MICROFLUIDIC SYSTEM FOR RAPID HBA1C MEASUREMENT

Ching-Chu Wu[1], Ko-Wei Chang[1], Hsin-I Lin[2], Shu-Chu Shiesh[2] and Gwo-Bin Lee[1*]
[1]Department of Power Mechanical Engineering, National Tsing Hua University, Hsinchu, Taiwan 300
[2]Department of Medical Laboratory Science and Biotechnology, National Cheng Kung University, Tainan, Taiwan 701

ABSTRACT

Diabetes mellitus (DM) is one of the most prevalent diseases and easily lead to serious complications. Glycosylated hemoglobin (HbA1c), an emerging biomarker for reliable monitoring of DM is commonly detected by bench-top immunoassays, which are labor-intensive and time-consuming. As an advance to existing methods, a microfluidic platform capable of performing automated immunoassay for HbA1c detection within 25 minutes is presented in this work. The microfluidic assay was able to detect HbA1c from human whole blood samples and delivered similar performance as its bench-top counterpart. The developed microfluidic chip is therefore promising for rapid detection of HbA1c for monitoring DM.

KEYWORDS: Diabetes mellitus, Microfluidics, Hemoglobin, HbA1c

INTRODUCTION

Diabetes mellitus (DM) affects nearly 10 percent of adults worldwide and has become one of the most prevalent diseases in the modern world. Often leading to serious complications such as cardiovascular diseases and nephropathy, diabetes therefore not only presents a health burden but also an economic burden. Toward the prevention and the proper treatment of diabetes, monitoring and thereby maintaining the blood glucose is critical. Although direct measurement of blood glucose using the glucose meter has been a ubiquitous approach, such measurement can easily fluctuate due to intake of food and drugs, as well as the physical condition of the patient. Recently, glycosylated hemoglobin (HbA1c) has emerged as an accurate indicator for diabetes [1]. It provides an average value of blood glucose over the previous 2-3 months. Existing techniques to measure HbA1c, such as immunoassays, however, are relatively expensive, labor-intensive and time-consuming. To tackle these problems and enable wide-spread use of HbA1c-based monitoring for diabetes, this study reports a microfluidic system that automatically detects and quantifies clinically-relevant HbA1c concentrations from human blood samples.

EXPERIMENTAL

In this work, a magnetic-bead-based, two-antibody immunoassay was realized in a microfluidic chip to achieve HbA1c detection. The schematic representation of the assay is depicted in Figure 1. A magnetically-modified capture antibody and an acridinium ester-labeled signaling antibody were used to capture and detect HbA1c. The chemiluminescence from acridinium ester was finally detected by a portable luminometer.

Figure 2(a) shows a photograph of the chip with dimensions of 3.8 cm × 6.8 cm and the components of microfluidic chip, which includes micropumps, micromixers, a microinjector, reservoirs, reaction chambers, microchannles, and normally-closed valves. Those components were controlled by electromagnetic valves and were regulated by a custom-made microcontroller. Figure 2(b) shows the exploded view of the chip, which consisted of a liquid channel layer, an air channel layer and a glass substrate.

Figure 3 shows a schematic illustration of the experimental procedure implemented on the integrated microfluidic chip. First, hemolysate acquired from treated human whole blood and anti-Hb antibody-coated magnetic beads were loaded into the device and mixed by the micromixer. Hb and HbA1c were captured by the magnetic beads and the interferent materials were washed away by the micropump. Then acridinium ester-labeled HbA1c antibodies were added into the chamber to bind with HbA1c, and unbound antibodies where washed away. Finally, H_2O_2 and NaOH were added to the sample and chemiluminescent signals were detected by a portable luminometer.

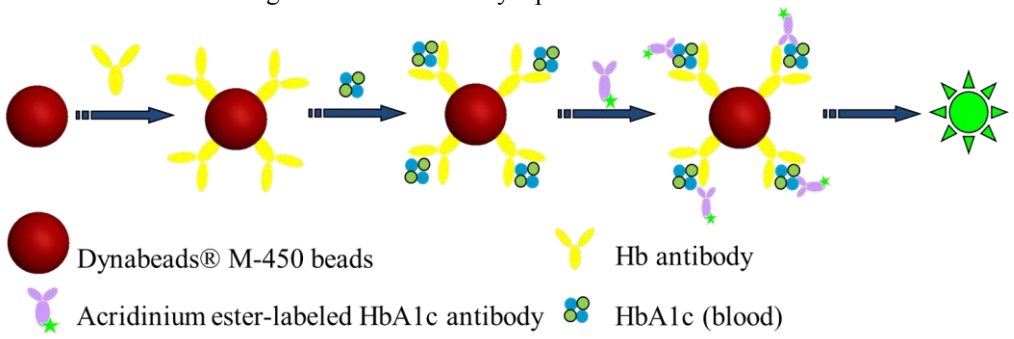


Figure 1: Schematic representation of the experiment protocol for the antibody-based detection of the developed method.

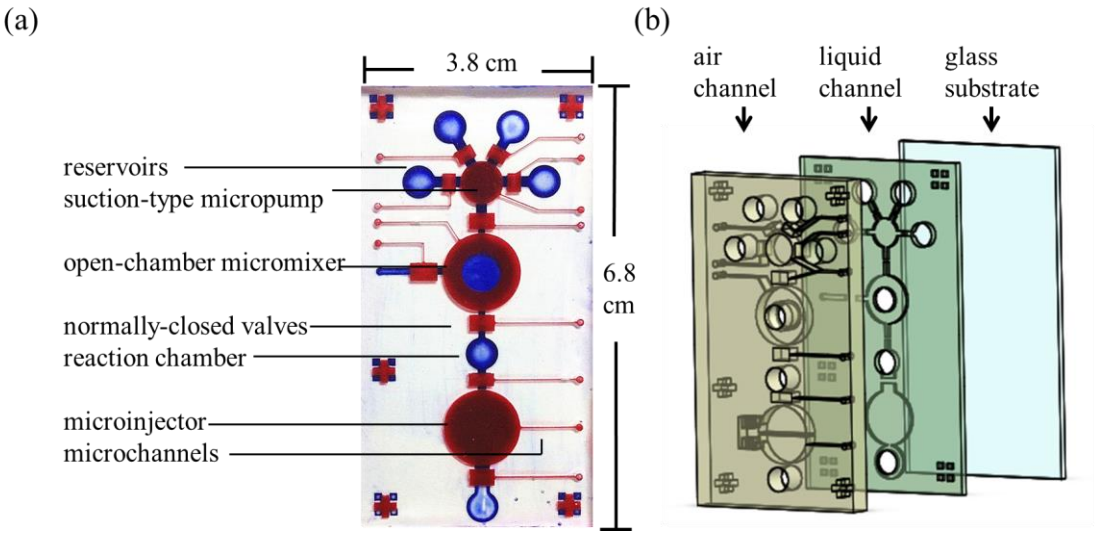
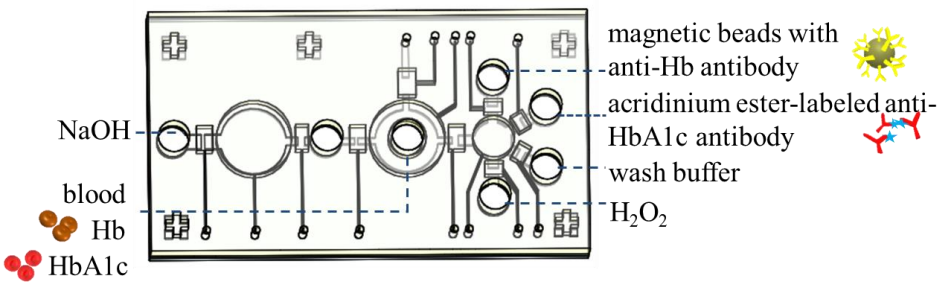


Figure 2: (a) A photograph and the microfluidic components, and (b) exploded view of the chip.



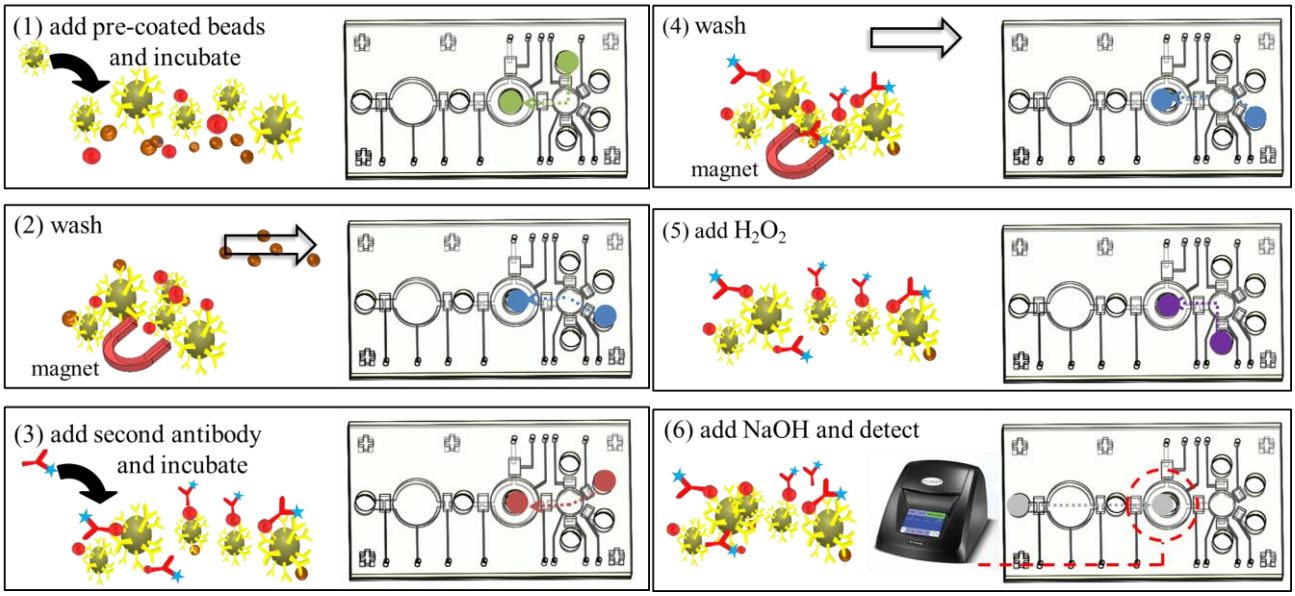


Figure 3: Schematic illustration of the experimental procedure implemented on the integrated microfluidic chip.

RESULTS AND DISCUSSION

Chemiluminescence signals at different HbA1c concentrations and the calibration curve of the HbA1c generated from the immunoassay performed with bench-top systems and with the microfluidic chip are shown in Figure 4 and Figure 5, respectively. Importantly, these results were obtained from human whole blood samples. Both calibration curves displayed a linear response. When compared to the bench-top-based detection, microfluidic-based detection demonstrated smaller variations in measurements, particularly at around 7% HbA1c – the typical threshold level for indicating diabetic patients. Furthermore, the microfluidic assay was completed in 25 minutes, which was much faster than the bench-top system (3.5 hours). Finally, the microfluidic assay required only half of the sample and reagent volumes as required by the bench-top system.

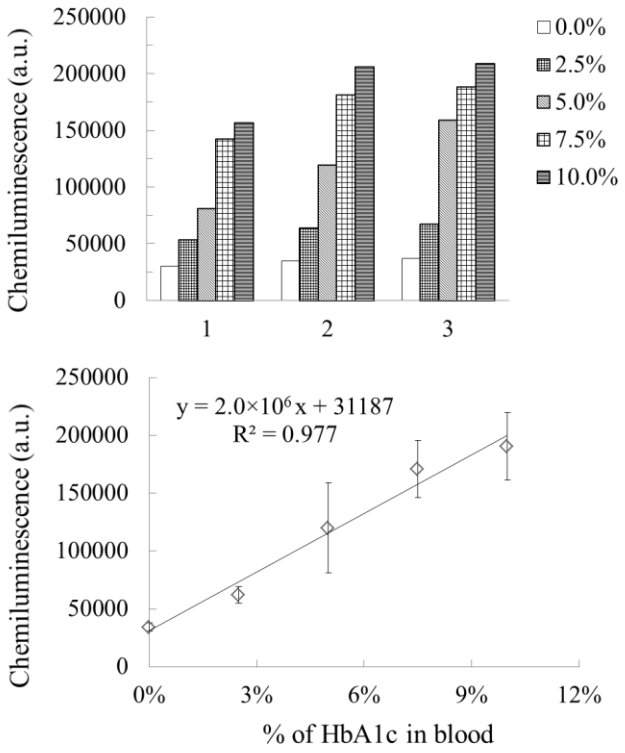


Figure 4: The HbA1c chemiluminescence signals at different level of HbA1c (top) and the calibration curve of the HbA1c generated from the immunoassay on bench experiments (bottom) for three times. Note that three consecutive measurements were performed.

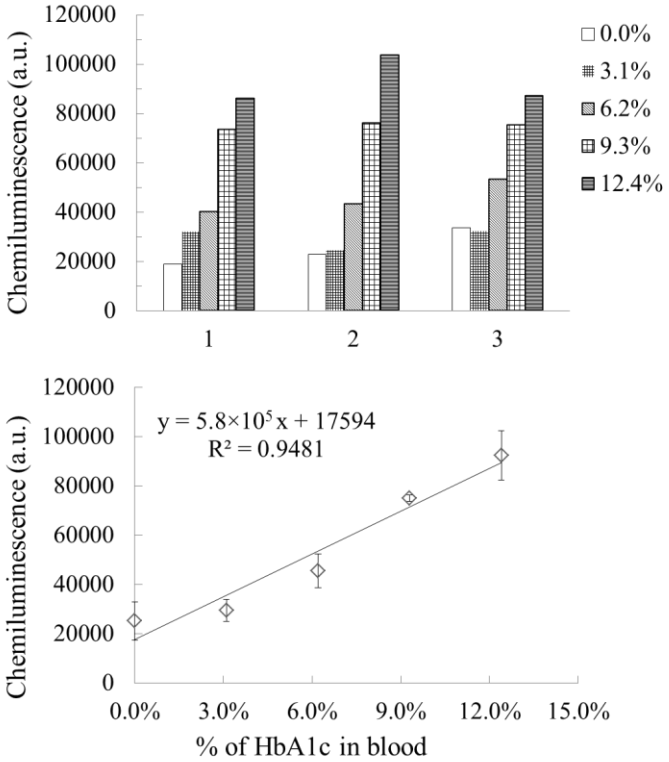


Figure 5: The HbA1c chemiluminescence signals at different level of HbA1c (top) and the calibration curve of the HbA1c generated from the immunoassay performed in the microfluidic system (bottom) for three times. Note that three consecutive measurements were performed.

CONCLUSION

In this work, an integrated microfluidic chip was developed to perform a magnetic-bead-based, two-antibody immunoassay for the detection of HbA1c in a rapid and automated fashion. The microfluidic assay was able to detect HbA1c from pretreated human blood samples and delivered similar analytical performances as conventional bench-top assays. Less reagents and samples were consumed for the entire process, leading to reduced cost. More importantly, the whole assay can be automatically performed in 25 minutes, which obviates manual operation and significantly reduce the assay time when compared with conventional bench-top assays (3.5 hours). The developed microfluidic chip is therefore promising for rapid detection of HbA1c from human whole blood samples.

ACKNOWLEDGEMENTS

The authors would like to thank the National Science Council in Taiwan for financial support (NSC 101-2218-E-007-006).

REFERENCES

[1] The Diabetes Control and Complications Trail Research Group, “The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus,” New England Journal of Medicine, 329, 977–986, 1993.

[2] W. B. Lee, Y. H. Chen, H. I. Lin, S. C. Shiesh, G. B. Lee, “An integrated microfluidic system for fast, automatic detection of C-reactive protein,” Sensors and Actuators B, 157, 710–721, 2011.

[3] C. J. Huang, H. C. Chien, T. C. Chou, G. B. Lee, “Integrated microfluidic system for electrochemical sensing of glycosylated hemoglobin,” Microfluid Nanofluid., 10, 37–45, 2011.

CONTACT

*Dr. Gwo-Bin Lee, Department of Power Mechanical Engineering, Institute of Biomedical Engineering and Institute of NanoEngineering and Microsystems, National Tsing Hua University, Hsinchu, Taiwan 300. Tel: + 886-3-5715131-33765; Fax: + 886-3-5722840; E-mail: gwobin@pme.nthu.edu.tw