DEVELOPMENT AND CHARACTERIZATION OF A GENERIC MICROFLUIDIC SUBSYSTEM TOWARD PORTABLE BIOCHEMICAL DETECTION

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Abstract

This paper presents the development of a generic microfluidic system toward portable biochemical detection. Microfluidic and electrochemical detection devices such as microvalves, flow sensors, biofilters, and immunosensors have been successfully developed and characterized in this work. A magnetic bio-bead approach has been adopted for both sampling and manipulating target biological molecules. The microfluidic and electrochemical immunosensing experiment results obtained from this work have shown that the biochemical sensing capability of the complete microfluidic subsystem is suitable for portable biochemical detection of bio-molecules.

Keywords: microfluidic system, biochemical detection, BioMEMS, biofilter

1. Introduction

In the past few years, a large number of microfluidic prototype devices and systems have been developed, specifically for biochemical warfare detection systems and portable diagnostic applications [1-2]. The BioMEMS team at the University of Cincinnati has been working on the development of a remotely accessible generic microfluidic system for biochemical and biomedical analysis, based on the concepts of both surface-mountable microfluidic motherboards and electrochemical detection techniques [1]. The limited goal of this work is to develop a generic MEMS-based microfluidic system and to apply the fluidic system to detect micro-organisms in liquid samples. Figure 1 illustrates the schematic diagram of a generic microfluidic system for biochemical detection using a magnetic bio-

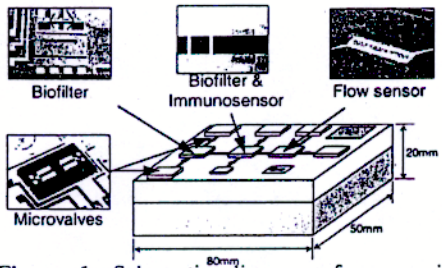

Figure 1. Schematic diagram of a generic microfluidic system for biochemical detection.

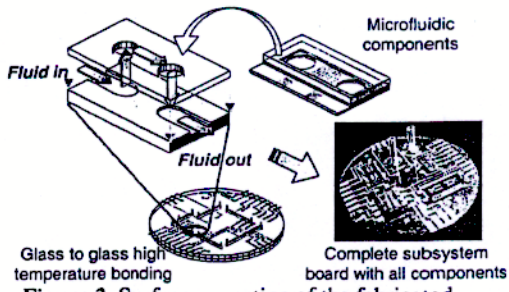

Figure 2. Surface-mounting of the fabricated microfluidic devices on a microfluidic subsystem.

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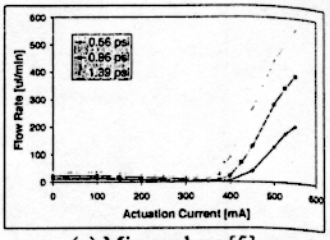
bead approach for both sampling and manipulating the target bio-molecules [3]. The analytical concept is based on an electrochemical immunoassay [4].

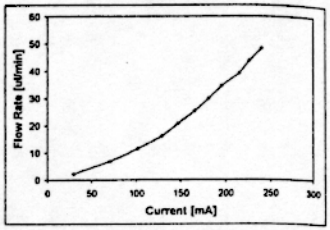
To realize the generic microfluidic system in this work, several new BioMEMS components have been explored for both microfluidic components such as valves, pumps, and flow sensors and for biochemical cells such as biofilters and biosensors. This paper describes the preliminary experimental results obtained from the realized BioMEMS microfluidic devices and biochemical cells, toward realizing a full electrochemical immunoassay-based remote biochemical sensor system.

2. Microfluidic Systems

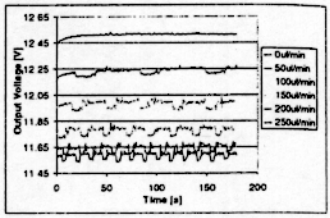
To realize the generic microfluidic subsystem, microfluidic and electrochemical detection devices such as microvalves [5], flow sensors [6], biofilters [3], and immunosensors [4] have been successfully fabricated and surface-mounted using a fluoropolymer bonding technique [7] on a microfluidic motherboard, which consists of microchannels and electrical interfaces to construct the generic microfluidic subsystem. The basic structural concepts of the fluidic interconnection and the realized generic microfluidic subsystem with all the microfluidic and immunosensing components are shown in Figure 2. The components have also been individually characterized and the results are shown in Figure 3.



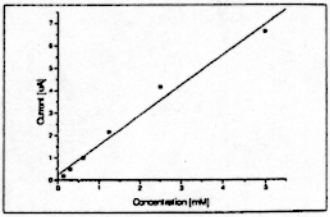
(a) Microvalves [5]



(b) Biofilter [3]



(c) Flow sensor [6]



(d) Immunosensor [4]

Figure 3. Characteristics of BioMEMS components.

3. Biochemical Detection Schemes

The assay being developed to detect target bio-molecule is a magnetic bead-based sandwich enzyme immunoassay through electrochemical detection using interdigitated electrode arrays (IDA) [4]. As described in Figure 4, the beads are coated with streptavidin and primary antibody is attached via labeled biotin. This general attachment chemistry makes the immunoassay easily extendible to any binding protein which is biotin labeled. The analyte is captured by a primary antibody on beads immobilized in the biofilter. The biofilter holds the beads in place magnetically while the sample is passing through the reactor. After the sampling step, a secondary antibody with enzyme label is added to form the sandwich. The beads are then released for transfer to a sensing chamber in which they are again trapped by a magnetic field and then, in the sensing chamber the enzyme substrate is added and the product is generated by redox cycling. After a period, the final quantification step is accomplished by electrochemical detection of the product as shown in Figure 5.

4. Results and Discussion

The assembled prototype system has been tested by flowing biofluids through the microchannels, for both flow monitoring and biosensing capability, which are believed as the most essential two functions of the system.

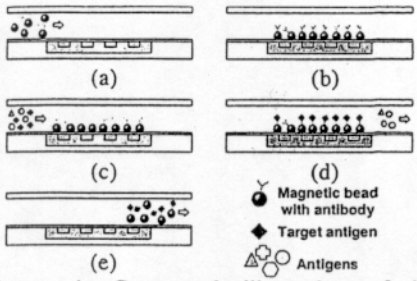


Figure 4. Conceptual illustration of bio-sampling procedure: (a) injection of magnetic beads; (b) separation and holding of beads; (c) flowing samples; (d) immobilization of target antigen; and (e) release to biosensor.

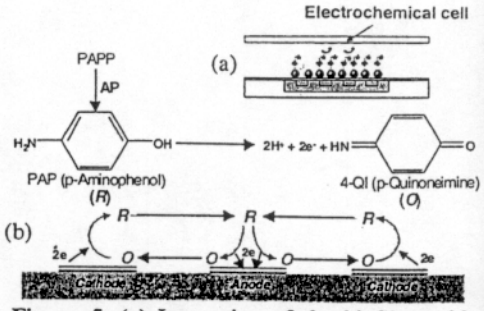
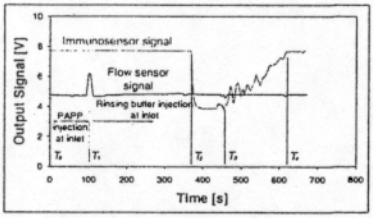


Figure 5. (a) Integration of the biofilter with detection components for electrochemical detection using enzyme as a label and (b) Detection principle of IDA and redox cycling

For reliable results from the immunosensor, it is necessary to ensure that reagents flowing sequentially across the immunosensor, do not come in contact with each other at all. When the solutions change, reagents in dead volumes "contaminate" the following reagent and eventually drop the sensitivity, requiring that the two reagents be separated by sufficient amount of buffer to ensure that no "contamination" of the reagents takes place. We have developed a technique using both the flow sensor and immunosensor to overcome this problem. To characterize the volume of rinsing fluid required the system is injected with DI water followed by an aliquot of PAPP solution which is a substrate for the immunoassay under development for this work. Figure 6 shows the measured output voltage of the liquid as a function of time, as the solution changes from DI water to PAPP solution then to DI water, at a flow rate of 50 µl/min. The time taken to restore the voltage allows us to calculate the amount of rinsing buffer required, ensuring complete separation of the reagents.



T_0: Reagent (PAPP) injected at the inlet
T_1: Rinsing buffer injection begins
(Reagent is traveling thought microchannels)
T_2: PAPP reached at the immunosensor
T_3: Rinsing buffer reached at the immunosensor and rinsing begins
T_4: Complete rinsing out and signal restored

Figure 6. Output of the immunosensor as a function of time. The time taken to reach the original voltage is indicative of the rinsing volume required.

The immunosensor has been also tested in the PC controlled meso-system, which is operated by commercially available microvalves and micropump. The microfabricated sensing chamber, which has a biofilter and an electrochemical immunosensor is connected to the valves and reservoirs to perform a magnetic bead-based sandwich immunoassay and the characteristics are shown in Figure 7. To ensure that the results of component evaluation on the meso-system will be valid on the microsystem, the cross-section of the tubes used in the meso-system has been kept similar to the cross-section of the microfluidic channels in the BioMEMS system.

5. Conclusions

The generic microfluidic subsystems toward portable bio/chemical detection have been developed and characterized for both microfluidic and electrochemical immunosensing

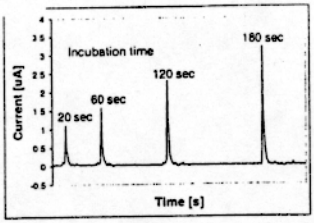


Figure 7. Representative results an immunoassay for different PAPP incubation times.

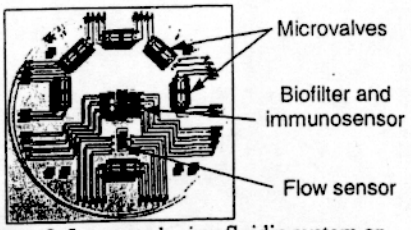


Figure 8. Integrated microfluidic system on 3-inch glass wafer.

aspects. Using the developed microfluidic devices which are surface-mounted on a microfluidic motherboard with electrochemical immunosensors, the microfluidic characterization technique has enabled us to understand and overcome the effects of dilution/mixing and dead volume in the microfluidic subsystems. Successful magnetic bead-based immunoassay results in the PC controlled meso-system have also been attained with the microfabricated biofilters and electrochemical immunosensors. Based on the results achieved from this work, a microprocessor controllable generic microfluidic subsystem for portable biochemical detection of bio-molecules has been realized as shown in Figure 8 and the system is now under characterization.

Acknowledgements

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References

1. C. H. Ahn, H. T. Henderson, W. R. Heineman, and H. B. Halsall, "Development of a Generic Microfluidic System for Electrochemical Immunoassay-Based Remote Bio/Chemical Sensors", Proceedings of the Micro Total Analysis Systems '98 (μ-TAS '98) Workshop, pp. 225-230, 1998.
2. C. Bisson, J. Campbell, R. Cheadle, M. Chomiak, J. Lee, C. Miller, C. Milley, P. Pialis, S. Shaw, W. Weiss, and C. Widrig, "A Microanalytical Device for the Assessment of Coagulation Parameters in Whole Blood", Technical Digest of Solid-State Sensor and Actuator Workshop 1998, pp. 1-6, 1998.
3. J.-W. Choi, S. Bhansali, C. H. Ahn, and H. T. Henderson, "A New Magnetic Bead-Based Filterless Bio-Separator with Planar Electromagnet Surfaces", Proceedings of Eurosensors XIII, pp. 713-716, 1999.
4. W. R. Heineman, H. B. Halsall, M. Cousino, C. A. Wijayawardhana, S. Purushothama, S. Kradtap, K. Schlueter, J.-W. Choi, C. H. Ahn, and H. T. Henderson, "Electrochemical Immunoassay with Microfluidic Systems," PITTCON '99, Orlando, FL, March 7-12, 1999.
5. D. J. Sadler, K. W. Oh, C. H. Ahn, S. Bhansali, and H. T. Henderson, "A New Magnetically Actuated Microvalve for Liquid and Gas Control Applications", Proceedings of Transducers '99, pp. 1812-1815, 1999.
6. N. Okulan, H. T. Henderson, and C. H. Ahn, "A New Pulsed-Mode Micromachined Flow Sensor for an integrated Microfluidic System", Technical Digest of Solid-State Sensor and Actuator Workshop 1998, pp. 320-323, 1998
7. A. Han, K. W. Oh, S. Bhansali, H. T. Henderson, and C. H. Ahn, "A Low Temperature Biochemically Compatible Bonding Technique Using Fluoropolymers for Biochemical Microfluidic Systems", Proceedings of IEEE MEMS '99, pp. 414-418, 2000.