AN INTEGRATED MICROFLUIDIC BIOCHEMICAL DETECTION SYSTEM WITH MAGNETIC BEAD-BASED SAMPLING AND ANALYSIS CAPABILITIES

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ABSTRACT

This paper presents the development and characterization of an integrated microfluidic biochemical detection system for fast and low volume immunoassays using magnetic beads, which are used as both immobilization surfaces and bio-molecule carriers. Magnetic bead-based immunoassay, as a typical example of biochemical detection and analysis, has been successfully performed on the integrated microfluidic biochemical analysis system that includes a surface-mounted biofilter and immunosensor on a glass microfluidic motherboard. Total time required for full immunoassay was less than 20 minutes including sample incubation time and sample volume wasted was less than 50 μl during five repeated assays. Fast and low volume biochemical analysis has been successfully achieved with the developed biofilter and immunosensor, which is integrated to microfluidic system.

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 detection and biomedical analysis, based on the concepts of both surface-mountable microfluidic motherboards and electrochemical detection techniques [1]. Through these papers, basic concepts and development of a several type of generic microfluidic subsystems were demonstrated focusing on microfluidic performances. In this paper, however, in addition to development of a new integrated microfluidic biochemical detection and analysis system, a full magnetic bead-based immunoassay is reported as a typical example of biochemical analysis.

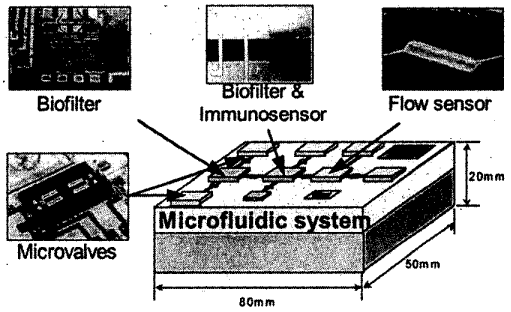

Figure 1. Schematic diagram of a generic microfluidic system for biochemical detection.

The limited goal of this work is to develop a generic MEMS-based microfluidic system and to apply the fluidic system to detect bio-molecules such as specific proteins and/or antigens in liquid samples. Figure 1 illustrates the schematic diagram of a generic microfluidic system for biochemical detection using a magnetic bead approach for both sampling and manipulating the target bio-molecules [3]. The analytical concept is based on sandwich immunoassay and electrochemical detection [4] as illustrated in Figure 2.

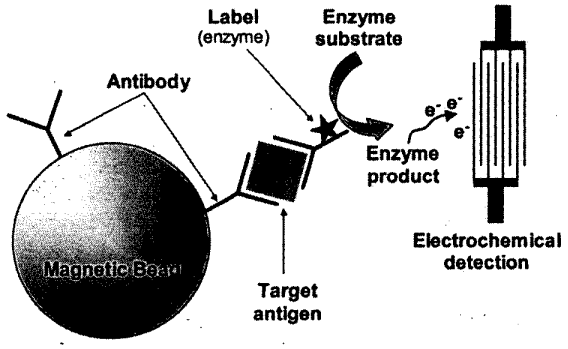

Figure 2. Analytical concept based on sandwich immunoassay and electrochemical detection.

Magnetic beads are used as both substrate of antibodies and carriers of target antigens. A simple concept of magnetic bead-based bio-sampling with electromagnet for the case of sandwich immunoassay is shown in Figure 3. Antibody coated beads are introduced on the electromagnet and separated by applying magnetic fields. While holding the antibody-coated beads, antigens are injected into the channel. Only target antigens are immobilized and thus, separated onto the magnetic bead surface due to antibody/antigen reaction. Other antigens get washed out with the flow. Next, enzyme-labeled secondary antibodies are

introduced and incubated, with the immobilized antigens. The chamber is, then, rinsed to remove all unbound secondary antibodies. Substrate solution, which will react with enzyme, is injected into the channel and the electrochemical detection is performed. Finally the magnetic beads are released to waste chamber and the bio-separator is ready for another immunoassay.

This paper describes the magnetic bead-based sampling and immunoassays obtained from the realized integrated microfluidic biochemical detection system, which includes a biofilter [3], an immunosensor [4], and microfluidic channels.

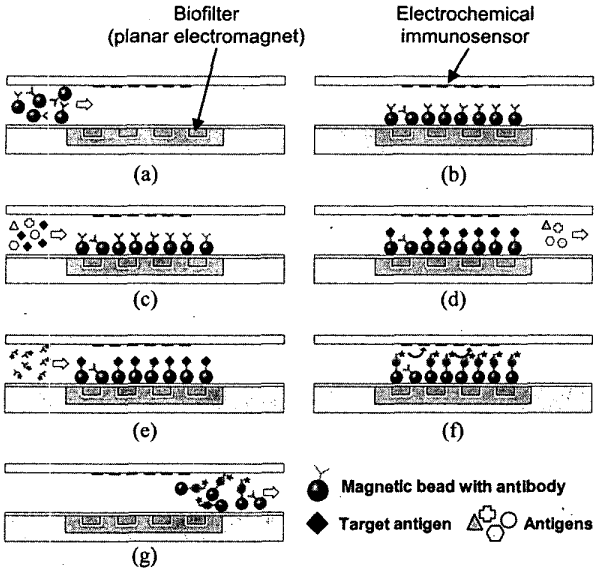

Figure 3. Conceptual illustration of bio-sampling and immunoassay procedure: (a) injection of magnetic beads; (b) separation and holding of beads; (c) flowing samples; (d) immobilization of target antigen; (e) flowing labeled antibody; (f) electrochemical detection; and (g) washing out magnetic beads and ready for another immunoassay.

MICROFLUIDIC COMPONENTS AND SYSTEM

For the successful immunoassay, the biofilter [3] and the immunosensor was fabricated separately and integrated together as shown in Figure 4. The reaction and sensing chamber volume is about 750 nl. The integrated biofilter and immunosensor was surface-mounted using a fluoropolymer bonding technique [6] on a microfluidic motherboard, which contains microchannels fabricated by glass etching and glass to glass direct bonding technique. All microchannels are 400 μm wide and 100 μm deep. The basic structural concept of the fluidic interconnection is illustrated in Figure 5.

Using computational fluid dynamics (CFD) simulation, microchannel structures were designed to minimize interactions between biochemical reagents to increase detection and analysis sensitivity. Each inlets and outlets were connected to sample reservoirs through custom-designed microvalves. Figure 6 shows the integrated microfluidic biochemical detection system for magnetic bead-based immunoassay.

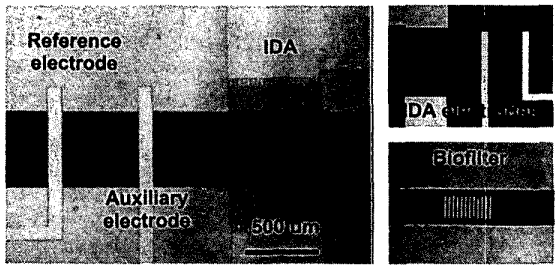

Figure 4. Photograph of the fabricated biofilter and immunosensor.

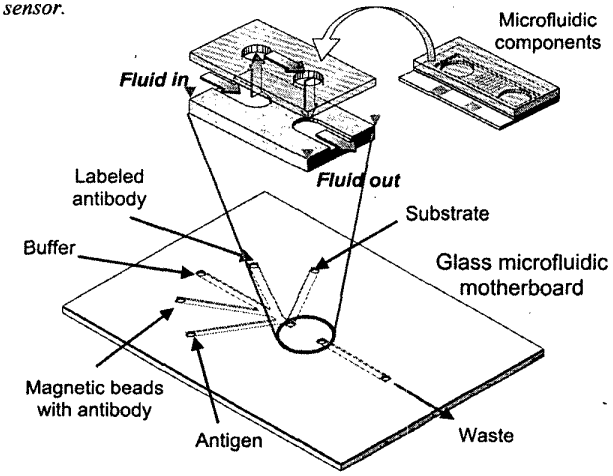

Figure 5. Illustration of microfluidic interconnection and surface-mounting technique.

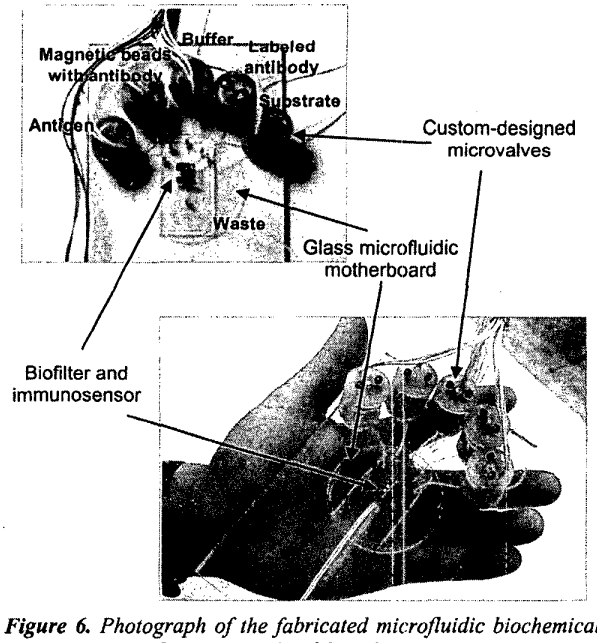

Figure 6. Photograph of the fabricated microfluidic biochemical detection system for magnetic bead-based immunoassay.

ELECTROCHEMICAL DETECTION SCHEMES

Alkaline phosphatase (AP) and p-aminophenyl phosphate (PAPP) was chosen as enzyme and electrochemical substrate. Alkaline phosphatase makes PAPP turn into its electrochemical product, p-aminophenol (PAP). By applying potential, PAP gives electrons and turns into 4-quinoneimine (4QI), which is oxidant form of PAP. Figure 7 illustrates the electrochemical detection principle.

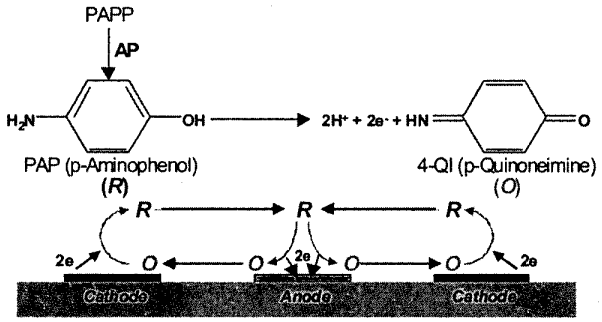

Figure 7. *Enzymatic kinetics for electrochemical detection of the immunosensor.*

MAGNETIC BEAD-BASED IMMUNOASSAY

After fluidic sequencing test, full immunoassays were performed in the integrated microfluidic system to prove magnetic bead-based biochemical detection and sampling function.

Magnetic beads (Dynabeads® M-280, Dynal Biotech Inc., shown in Figure 8) coated with biotinylated sheep anti-mouse Immunoglobulin G (IgG) were injected into the reaction chamber and separated on the surface of the biofilter by applying magnetic fields. While holding the magnetic beads, antigen (mouse IgG) was injected into the chamber and incubated. Then secondary antibody with label (rat anti-mouse IgG conjugated alkaline phosphatase) and electrochemical substrate (PAPP) to alkaline phosphatase was sequentially injected and incubated to ensure production of PAP. Electrochemical detection using an amperometric time-based detection method was performed while incubation. After detection, magnetic beads with all reagents were washed away and the system is ready for another immunoassay. The sequence used for the immunoassay is summarized in Table 1. This sequence was repeated for every new immunoassay. The flow rate was set to 20 µl/min in every step.

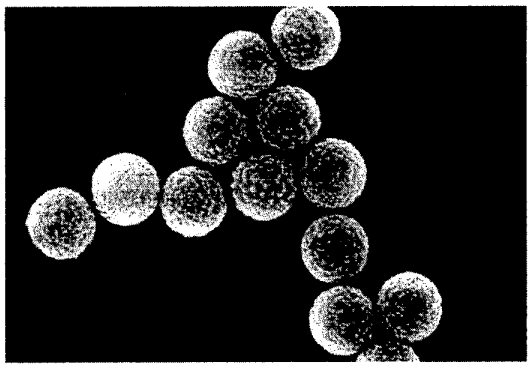

Figure 8. *Magnetic beads (Dynabeads® M-280. Photograph was provided by Dynal Biotech Inc.). Diameter of the bead is 2.8 µm.*

Table 1. *Sequence of the magnetic bead-based immunoassay.*

1)	Injection of primary antibody coated magnetic beads (biotinylated sheep anti-mouse IgG on magnetic beads) for 2 minutes
2)	Flowing buffer for 30 seconds
3)	Injection of antigen (mouse IgG) for 30 seconds
4)	Incubation for 5 minutes
5)	Flowing buffer for 30 seconds
6)	Injection of labeled antibody (alkaline phosphatase labeled rat anti-mouse IgG) for 30 seconds
7)	Incubation for 5 minutes
8)	Flowing buffer for 30 seconds
9)	Injection of substrate (PAPP) for 30 seconds
10)	Incubation for 5 minutes
11)	Detection for 1 minute
12)	Flushing everything out
13)	The system is ready for another assay
14)	Assay time: *Less than 20 minutes at 20 ul/min of flow rate*

EXPERIMENTAL RESULTS AND DISCUSSION

Before the actual magnetic bead-based immunoassays, the electrochemical immunosensor was tested and calibrated by flowing PAP solution. The calibration curve of the electrochemical immunosensor for different PAP concentration is shown in Figure 9.

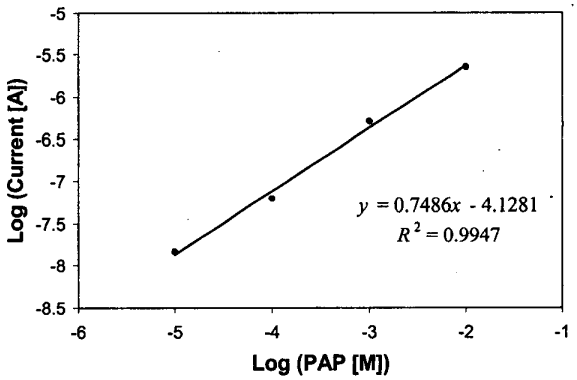

Figure 9. *Calibration curve of the electrochemical immunosensor for PAP solution, which is enzyme product.*

After calibration of the electrochemical immunosensor, full immunoassays were performed following the sequence in Table 1 for different antigen concentration: 50, 75, 100, 250, and 500

ng/ml. Concentration of primary antibody coated magnetic beads and conjugated secondary antibody was 1.02×10^7 beads/ml and 0.7 μg/ml, respectively. Immunoassay results for different antigen concentration are shown in Figure 10.

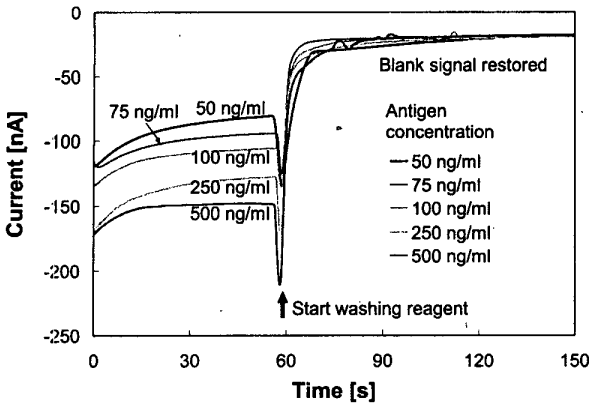

Figure 10. *Immunoassay results measured by amperometric time-based detection method. Immunoreactant consumed during one immunoassay was 10 μl (20 μl/min × 30 seconds) and total assay time was less than 20 minutes including all incubation and detection steps.*

Immunoreactant consumed during one immunoassay was 10 μl (20 μl/min × 30 seconds) and total assay time was less than 20 minutes including all incubation and detection steps. The assay time and volume depends on flow rate and incubation time, so the optimization of the immunoassay condition with microprocessor control is currently being investigated.

Reproducibility of the system was also tested through four times of repeated immunoassay for 100 ng/ml of antigen concentration and the results are shown in Figure 11.

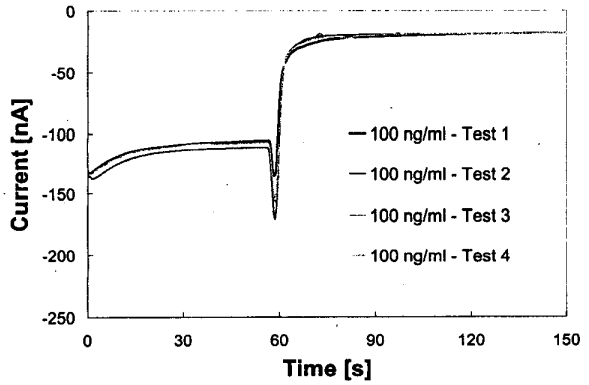

Figure 11. *Four repeated immunoassays with 100 ng/ml of antigen concentration.*

CONCLUSIONS

The integrated microfluidic biochemical detection system has been successfully developed and fully tested for fast and low volume immunoassays using magnetic beads, which are used as both immobilization surfaces and bio-molecule carriers. Magnetic bead-based immunoassay, as a typical example of biochemical detection and analysis, has been performed on the integrated microfluidic biochemical analysis system that includes a surface-mounted biofilter and immunosensor on a glass microfluidic motherboard. Protein sampling capability has been demonstrated by capturing target antigens.

The methodology and system, which has been developed in this work, can be also applied to generic bio-molecule detection and analysis systems by replacing antibody/antigen with appropriate bio receptors/reagents such as DNA fragments or oligonucleotides for the application to DNA analysis and/or high throughput protein analysis.

ACKNOWLEDGEMENT

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