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Magnetic Bead-Based Immunoassay on a Microfluidic Lab-on-a-Chip

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Abstract

This paper presents a basic concept of lab-on-a-chip systems and their advantages in chemical and biological analyses. In addition, magnetic bead-based immunoassay on a microfluidic system is also presented as a typical example of lab-on-chip systems. Rapid and low volume immunoassays have been successfully achieved on the demonstrated lab-on-a-chip using magnetic beads, which are used as both immobilization surfaces and biomolecule carriers. Total time required for an immunoassay was less than 20 minutes including sample incubation time, and sample volume wasted was less than 50 μl during five repeated assays. Lab-on-a-chip is becoming a revolutionary tool for many different applications in chemical and biological analysis due to its fascinating advantages (fast and low cost) over conventional chemical or biological laboratories. Furthermore, simplicity of lab-on-a-chip systems will enable self-testing capability for patients or health consumers overcoming space limitation.

I. Introduction

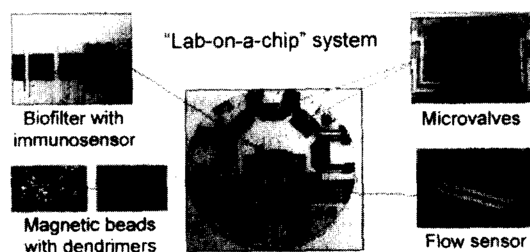
Recent development in MEMS(Microelectromechanical Systems) has brought a new and revolutionary tool in biological or chemical applications, which is named "lab-on-a-chip". New terminology, such as micro total analysis systems and lab-on-a-chip, was introduced in the past decade and many prototype systems have been reported[1-5].

The idea of lab-on-a-chip is basically to reduce biological or chemical laboratories into a microscale system in hand-held size or smaller. Lab-on-a-chip systems can be made out of silicon, glass, and polymeric materials and the typical microfluidic channel dimensions are in the range of several 10-100 μm. Liquid samples or reagents can be transported through the microchannels from reservoirs to reactors using pumping the liquids by electrokinetic, magnetic, or hydrodynamic methods. Fluidic motions or biochemical reactions also can be monitored using various sensors, which often serve for biochemical detection of products.

There are many advantages using lab-on-a-chip over conventional chemical or biological laboratories. One of the impor-

tant advantages lies on low cost. Many reagents and chemicals used in biological and chemical reaction are expensive, so the prospect of using very small amounts (in micro-to nano-liter range) of reagents and chemicals for an application is very appealing. Another advantage is that lab-on-a-chip requires very small amounts of reagents/chemicals, which enables rapid mixing and reaction because biochemical reaction is mainly involved in diffusion of two chemical or biological reagents and microscale fluidics reduces diffusion time as increases reaction probabilities. In practical terms, reaction products can be produced in a matter of seconds/minutes compared with laboratory scale taking hours or even days. In addition, lab-on-a-chip systems minimize harmful byproducts since their volume is so small. Complex reactions of many reagents could happen on lab-on-a-chip that has ultimate potential in DNA analysis, biochemical warfare agent detection, biological cell/molecule sorting, blood analysis, drug screening/development, combinatorial chemistry, and protein analysis.

In this paper, a hand-held microfluidic immunoassay system for biochemical warfare detection[5], which was developed by BioMEMS team at the University of Cincinnati, will be described as a typical example of lab-on-a-chip system as shown in ⟨Figure 1⟩.



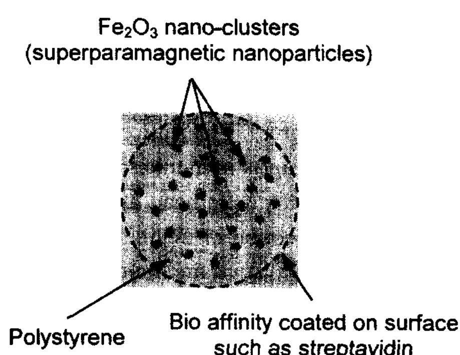
⟨Figure 1⟩ An example of lab-on-a-chip systems : A Generic Microfluidic System for Electrochemical Immunoassay-Based Remote Bio/Chemical Sensors[5].

II. Magnetic Beads in Lab-on-a-Chip

The basic concept in magnetic separations in biotechnologies including lab-on-a-chip systems is to selectively bind the biomaterial of interest, such as a specific cell, protein, or DNA fragments, to a magnetic particle and then separate it from its surrounding matrix using a magnetic field for manipulation or purification of biological cells/molecules. Magnetic beads of iron oxide (Fe_2O_3 or Fe_3O_4) with diameters ranging from a few nanometers to a few micrometers are typically used for such separations. These magnetic particles are called "superparamagnetic" particles, meaning that they are attracted to a magnetic field but retain no residual magnetism after the field is removed. The material, which is halfway between ferromagnetic and paramagnetic property, has superparamagnetism. As the size of magnetic elements scales below a few tens of nanometer range, a superparamagnetic phase emerges in which the room temperature thermal energy overcomes the magnetostatic energy well of the element, resulting in zero hysteresis[6]. In other words, although the element itself is a single-domain ferromagnet, the ability of an individual magnetic domain to store magnetization orientation information is lost when its di-

mension is below a threshold. Therefore, suspended superparamagnetic particles tagged to the biomaterial of interest can be removed from a matrix using a magnetic field, but they do not agglomerate or stay suspended in the solution after removal of the magnetic field. If the magnetic components (generally iron oxide) are small enough, they will respond to a magnetic field but are incapable of becoming independently magnetic. This is important, as it results in particles that are attracted to a magnetic field yet lose all attraction for each other in the absence of a magnetic field-allowing efficient separation and complete resuspension. 〈Figure 2〉 illustrates a general structure of magnetic beads that contain superparamagnetic nanoparticles in a polymeric shell.

The most attractive advantage of the magnetic separation technique in biochemistry and biotechnology is ease of manipulation of bio-molecules, which are immobilized on magnetic particles. Once target biological cells or molecules are immobilized on magnetic particles, the target bio-molecules can be separated from a sample solution, flexibly manipulated in various reagents, and easily transported to a desired location by controlling magnetic fields produced from a permanent magnet or an electromagnet. Another advantage is large surface area of immobilization substrate that results high population of target biological molecules due to large binding site and high detection signal.

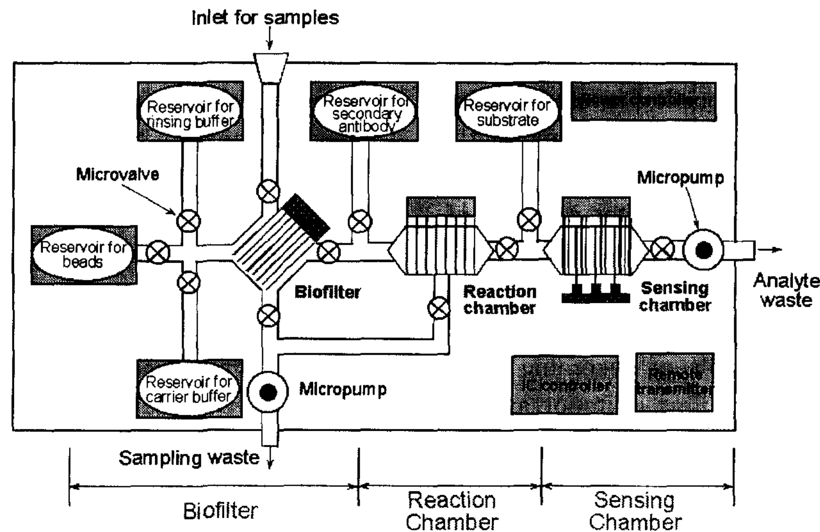


〈Figure 2〉 General structure of superparamagnetic beads. Surface of magnetic beads can be coated with various bio affinity groups depending on applications.

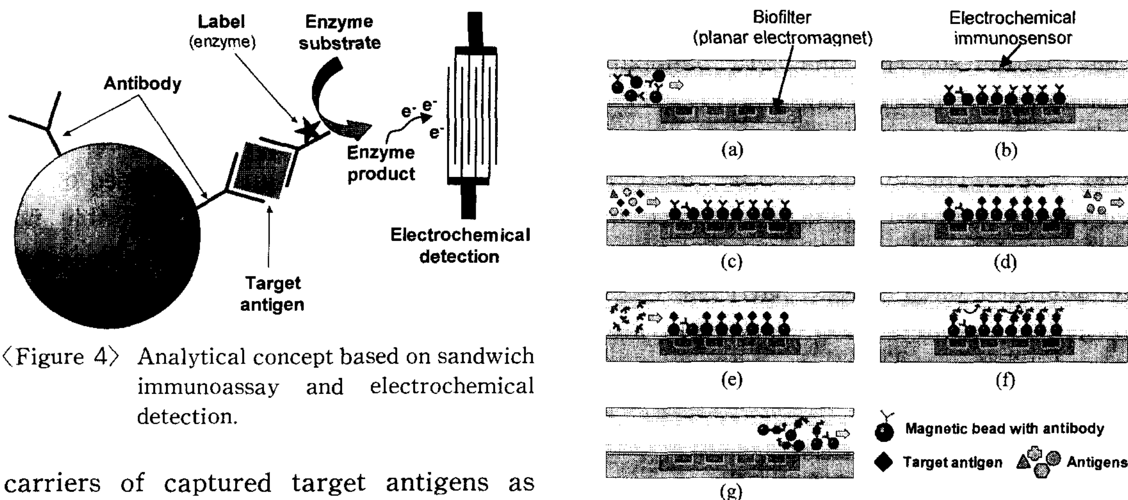
Ⅲ. Lab-on-a-Chip Immunoassay System Based on Magnetic Beads Approach

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, sandwich immunoassays, and electrochemical detection techniques[7]. The limited goal of this work was to develop a generic microfluidic lab-on-a-chip system and to apply the microfluidic system to detect bio-molecules such as specific proteins and/or antigens in liquid samples. 〈Figure 3〉 illustrates the schematic diagram of a generic microfluidic system for biochemical detection using a magnetic bead approach for both sampling and manipulating the target biomolecules. The analytical concept is based on sandwich immunoassay and electrochemical detection as illustrated in 〈Figure 4〉.

Magnetic beads are used as the solid phase for the capture of antibodies, and as



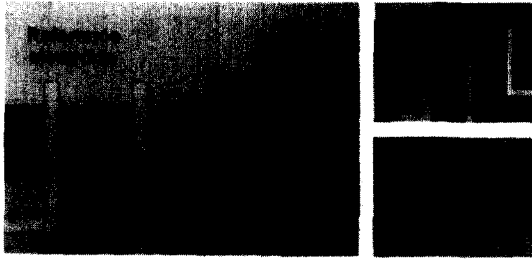
〈Figure 3〉 Schematic diagram of a generic microfluidic lab-on-a-chip system for biochemical detection.



〈Figure 4〉 Analytical concept based on sandwich immunoassay and electrochemical detection.

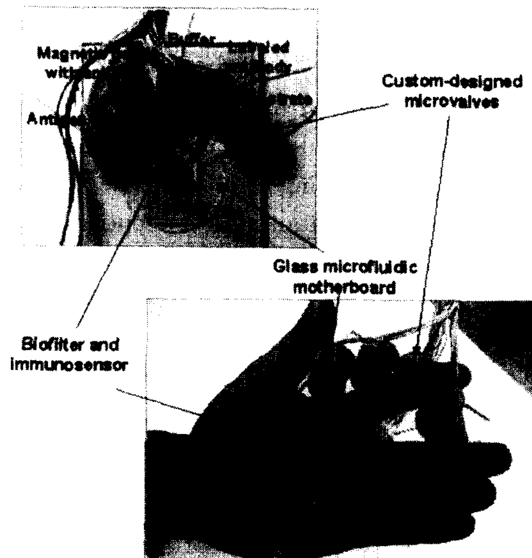
〈Figure 5〉 Conceptual illustration of bio-sampling and immunoassay procedure using magnetic bead approach :
(a) injection of magnetic beads ;
(b) separation and holding of beads ;
(c) flowing samples ;
(d) immobilization of target antigen;
(e) flowing labeled antibody ;
(f) electrochemical detection ; and
(g) washing out magnetic beads and ready for another immunoassay.

carriers of captured target antigens as mentioned above. A simple concept of magnetic bead-based bio-sampling with electromagnet for the case of sandwich immunoassay is shown in 〈Figure 5〉. Antibody coated beads are introduced to the electromagnet and separated by applying a magnetic field. 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



〈Figure 6〉 Microphotograph of the integrated biofilter and biosensor as a part of the lab-on-a-chip system. The volume of the fluidic chamber for biofiltration, reaction, and detection was calculated to 750 nl.

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



〈Figure 7〉 Photograph of the fabricated microfluidic lab-on-a-chip system for magnetic bead-based immunoassay.

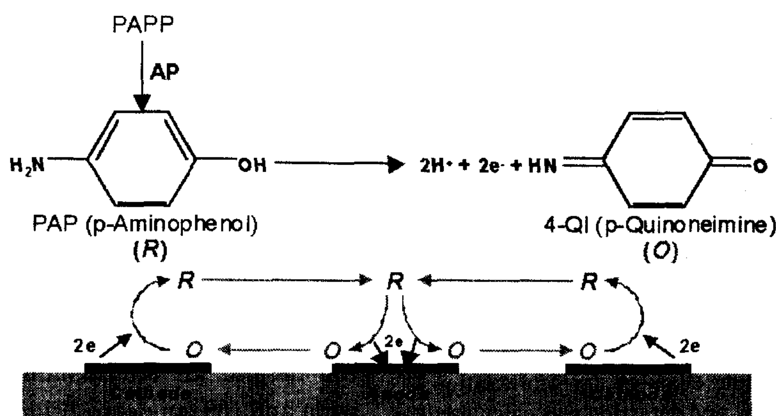
electrochemical detection is performed. Finally the magnetic beads are released to the waste chamber and the bio-separator is ready for another immunoassay.

For the successful immunoassay, the biofilter and the electrochemical sensor were fabricated separately and integrated together as shown in 〈Figure 6〉[8]. The reaction and sensing chamber volume is 750 nl and all microchannels are 400 μm wide and 100 μm deep. 〈Figure 7〉 shows the integrated lab-on-a-chip system for magnetic bead-based immunoassay.

Ⅳ. Magnetic Bead-Based Immunoassay

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[9]. 〈Figure 8〉 illustrates the electrochemical detection principle.

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.) 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



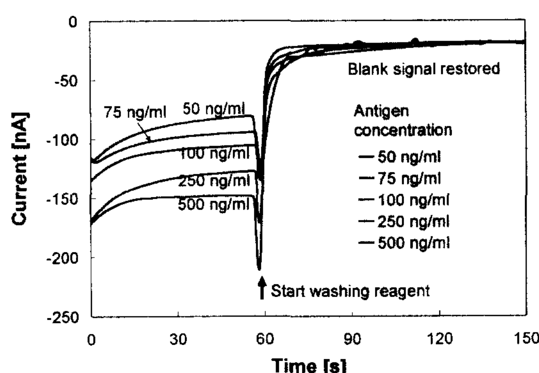
〈Figure 8〉 Enzymatic kinetics for electrochemical detection of the immunosensor.

〈Table 1〉 Sequence of the magnetic bead-based immunoassay on the developed lab-on-a-chip.

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
Assay time : *Less than 20 minutes at 20 ul/ min of flow rate*

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.

After calibration of the electrochemical immunosensor, full immunoassays were performed following the sequence stated above 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 9〉. Immunoreactant consumed during one immunoassay was 10 μl and total assay time was less than 20 minutes including all incubation and detection steps.



〈Figure 9〉 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.

V. Conclusion

The integrated microfluidic biochemical detection system has been demonstrated as a typical example of lab-on-a-chip systems. Rapid and low volume immunoassays have been successfully achieved on the demonstrated lab-on-a-chip using magnetic beads, which are used as both immobilization surfaces and bio-molecule carriers. Protein sampling capability has been demonstrated by capturing target antigens. The microfluidic lab-on-a-chip system and magnetic bead capture methodology, which has been reviewed in this paper, can also be applied to generic biomolecule detection and analysis systems by replacing antibody/antigen with appropriate bio receptors/reagents such as DNA fragments or oligonucleotides for detection and analysis of a wide variety of biological materials as well as to DNA analysis and high throughput protein analysis.

Lab-on-a-chip is becoming a revolutionary tool for many different applications in chemical and biological analysis due to its fascinating advantages (fast and low cost) over conventional chemical or biological laboratories. Furthermore, simplicity of lab-on-a-chip systems will enable self-testing capability for patients or health consumers overcoming space limitation.

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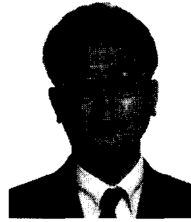

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