MULTICHANNEL MICRO ELISA SYSTEM

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Abstract
ELISA system was integrated into a glass microchip. Antigen-antibody and enzyme reactions were performed on a surface of microbeads which were packed in the chip. By monitoring the enzyme reaction product with a thermal lens microscope, good calibration curve was obtained with short assay time. Moreover, to realize higher throughput assay, a semi-automatic ELISA system with a 32-ch microchip was designed and constructed.

Keywords: ELISA, immunoassay, multichannel system, thermal lens microscope

1. Introduction
An immunoassay, including a enzyme-linked immunosorbent assay (ELISA) is known as one of the most important analytical methods and widely used in clinical diagnoses, environmental analyses and general biosciences. The conventional immunoassay, however, requires a long time for assay. We developed a beads-bed immunoassay system integrated on a microchip (Fig. 1), which enabled successful

Fig. 1 Cross section of immunoassay microchip
reduction of the assay time from 2 days to 35 min and required simple and easy liquid handling operations for assay [1-3]. However, because the system involved troublesome thermal lens microscope (TLM) measurement of colloidal gold which was fixed on the surfaces of several beads for one assay, further improvement in measurement method was required for higher throughput assay and automation.

Here, to solve the problem, a microchip-based ELISA system was developed. In the system, peroxidase was used as a labeling material instead of colloidal gold, and then the enzyme reaction product which is dissolved in a solution can be simply monitored by a TLM.

2. Microchip fabrication

The ELISA microchip has inlet and outlet holes, reaction area with a dam for packing the microbeads, and detection area (Fig. 2). Microchannels were fabricated on a Pyrex glass substrate (7 cm x 3 cm) by a combination of photolithography and wet etching technique. A dam was fabricated by connecting the two disconnected channels by another short time wet etching. Typically, the channel width and depth were 210 μm and 100 μm, respectively, and the channel depth of the dam region was 10μm.
3. MicroELISA

The system was applied to assays of a heart failure marker peptide, BNP. Aliquot of microbeads (20 μm in diameter), which were pre-coated with a capture antibody followed by blocking with BSA, was packed in the microchip, and then a sample, a buffer, an antibody-peroxidase conjugate, and a buffer were injected from an inlet hole successively. After final washing procedure, substrate solutions, i.e. ABTS and H₂O₂, were injected from the inlets and the resulting enzyme reaction product was monitored by a TLM (excitation wavelength: 405 nm) at a downstream part of the microchannel.

After optimization of reaction conditions, i.e., flow rates, reaction temperatures, reaction time, and reagent concentrations, good calibration curve was obtained with short assay time (Fig. 3). Compared with the conventional method, the system achieved a successful reduction of the assay time from 20 h to 20 min and ~100 times lower determination limit. The micro ELISA system realized simpler, more precise, and more sensitive determination than the previous microchip method. We concluded that the system was practical and applicable to various fields such as clinical diagnoses and environmental analyses.

Fig. 3 Calibration curve of a heart failure marker, BNP

Fig. 4 Micro ELISA system
(A) semi-automatic analyzer
(B) 32-channel ELISA chip
4. Semi-automatic analyzer

To realize more than 10 times higher throughput assay than the conventional commercial automatic systems, semi-automatic ELISA system with 32-ch microchip was designed and constructed. The analyzer consists of microsyringe pumps, reagents container, capillary tubing, connectors, a thermal controller, a scanning TLM detector, and a computer for system operation and data acquisition and processes with a touch-panel display (Fig. 4). The system was design to perform 32 ELISA tests at a time. Performance of the microfluidic system and the scanning TLM detector has been examined. Scanning signals of colored standard solution (10^{-4} M Ni-phthalocyanine derivative) under flow conditions were shown in Fig. 5. Profiles and efficiency of the antigen-antibody reactions and an enzyme reaction were also investigated. We concluded that the system has enough performance to realize highly sensitive ELISA with remarkable high throughput.

References

