# High Sensitive Immunoassay using Antibody Immobilized Micro Capillaries Fabricated by Deep X-ray Lithography

Yuichi Utsumi, and Toshifumi Asano
Laboratory of Advanced Science and Technology for Industry, University of Hyogo,
Kamigori, Hyogo Pref., Japan
E-mail: utsumi@lasti.u-hyogo.ac.jp

This paper presents a high sensitive and rapid enzyme linked immunosorbent assay (ELISA) method using micro capillary bundle structure in which thousands of micro capillary are collected as a fluid control filter and 3D chemical reaction space. The high aspect ratio microfabrication processes brought by deep X-ray lithography using synchrotron radiation [1] allows new type of vertical chemical operation. In this paper, we describe the application of the newly-fabricated reactor stack of micro capillary bundle structure to the ELISA with high speed and sensitivity.

The fluid for ELISA analyte and reagents are transported through the PMMA (φ3mm) micro capillary bundle structure as shown in Fig.1. We estimated the fluidics behavior during vertical operations using computational fluid dynamics simulation "FLUENT". Figure 2 shows the calculated and measured dependence of the load threshold pressure on the capillary diameter at which the pneumatic transportation of the fluid from the upper unit reservoir to the lower unit reservoir starts. It is notable that the threshold of the load pressure for the pneumatic operation is fairly low (< 0.12 atom), suggesting the pressure loss during vertical fluid transportation restricted to be low[2].

Next, we found significant properties of liquid mixing, which occurs during the transportation of liquid through the capillary bundle structure. It is also suggested the liquid will be mixed drastically by repeating transportation from top to down and in reverse motion. In order to confirm this, we tried to apply the reactor to an enzyme reaction, and observe extent of reaction in real time using ultra violet (UV) absorption spectroscopy. It was confirmed experimentally that 2-hydroxymuconate semialdehyde by the enzyme (catechol 2,3-dioxygenase) catalyze the matrix (catechol) very rapidly at the initial stage of the reaction, which suggests the drastic effects of the mixing.

As a model of ELISA using the microreactor, we planed to detect mouse immunoglobulin (IgG) by the use of the antibody-antigen reaction on the inside wall of the capillary as shown in Fig. 3. By using this structure, the density of antibody immobilization drastically increase which will lead to the enhancement of the sensitivity of ELISA. The concentration of the mouse IgG and enzyme-labeled anti-mouse IgG were measured by UV absorption. We found that the mouse IgG (100 ng/ml) was quantitatively detected within 45 min of analytical period, which was about one third of the period required for the conventional method using micro titer plate. We fabricated stacked structure in which three vertical bio-reactors are integrated inside the each plate as shown in Fig.4. By using this integrated structure, we also succeeded to obtain calibration curve of Mouse IgG rapidly in one chemical unit operation.

We also adapted our proposed micro chemical reactor stack to the analysis of endocrine disrupting chemical using ELISA, and nonylphenol was used as the analyte. As shown in Fig.5, an unlabeled primary antibody (anti nonylphenol antibody) is coated onto the inside walls of the micro capillary bundle structure. This primary antibody is then incubated with nonylphenol and HRP(Horse radish peroxidase) conjugated nonylphenol. The HRP conjugated nonylphenol and nonhylphenol will bind to the primary antibody competitively. The labeled primary antibody is then developed with substrate and color change is measured. $B/B_0$ is the ratio of the analytical signal (absorbance) at the measured concentration of nonylphenol (B) to signal obtain in the absence of the analyte ($B_0$). As shown in Fig.6 detection limit was observed to be less than 0.1μg/l, which suggests the high sensitivity of the ELISA achieved by proposed microreactor stack. The detection limit of micro filter ELISA system will be more decreased if chemiluminescent substance is used.

References

[1]Y. Utsumi, T. Asano, Y. Ukita, K. Matsui, M. Takeo, S. Negoro, Jpn. J. Appl. Phys., [44], 5707 (2005)
[2]Y. Utsumi, T. Kishimoto, J.Vac.Sci.Technol. B, [23], 2903, (2005)

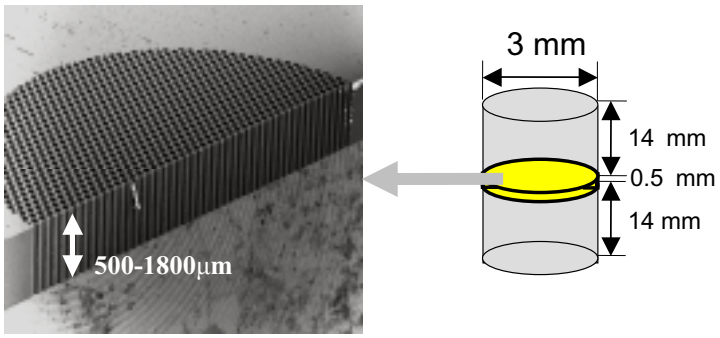


**Fig. 1** Photographs and schimatic illustration of micro capillary bundle structure fabricated by deep x-ray lithography in which thousands of micro capillary are collected. The liquid can be selectively transferred by air pressure from upper vessel to lower, and vice versa

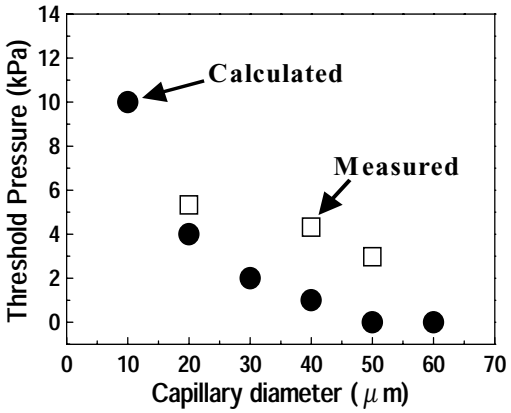


**Fig. 2** The calculated and measured dependence of the load threshold pressure on the capillary diameter

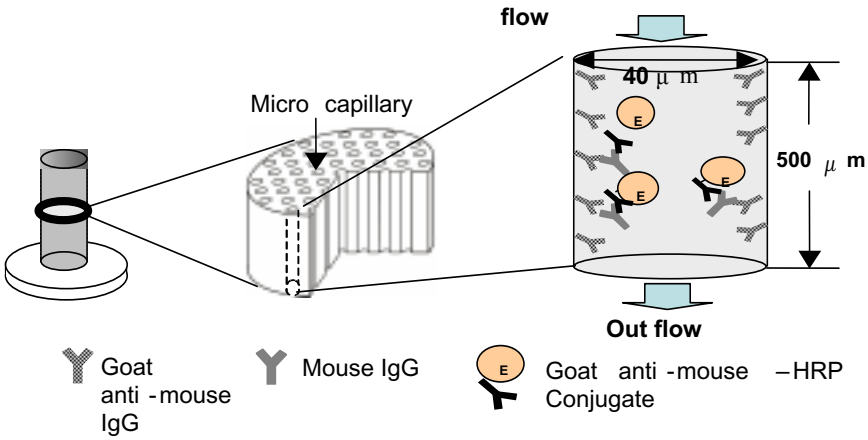


**Fig. 3** Schematic illustration of the ELISA method on micro fluid filter. We immobilized the goat anti-IgG antibody on the inside wall of the micro capillary bundle structure, and assayed the mouse IgG by ELISA using anti-IgG antibody/ peroxidase conjugate

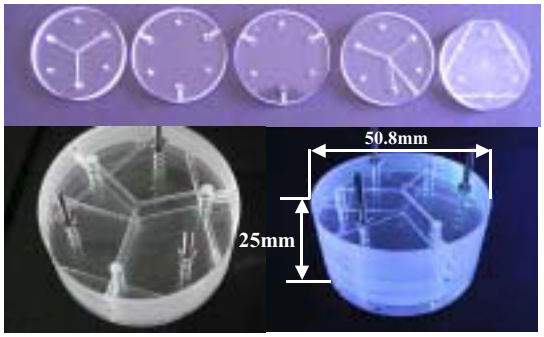


**Fig. 4** Photographs of integrated stacked structure consists from three micro reactor

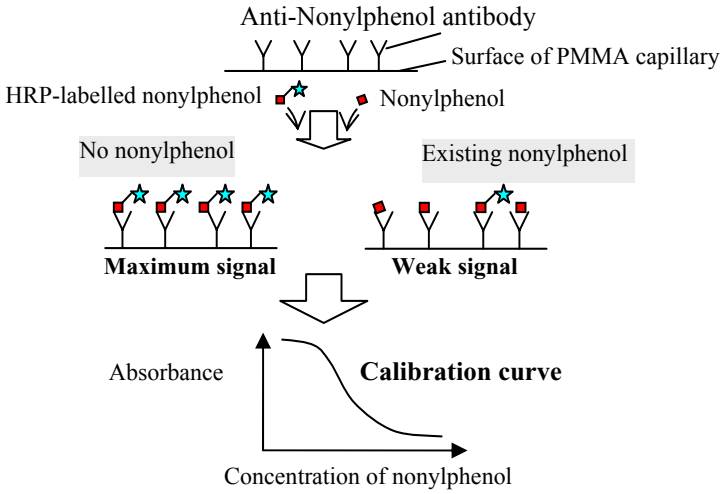


**Fig. 5** Principle of nonylphenol assay based on competitive ELISA method

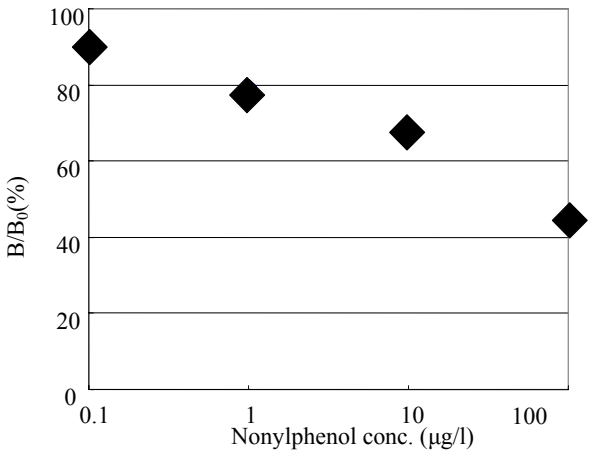


**Figure 6** Calibration curve for nonylphenol concentration measurement using ELISA method