MICROCHAMBER ARRAY FOR IMMUNOSENSOR APPLICATIONS

Yuji Murakami, Takayuki Kikuchi, Masahiro Yanase, Hidenori Nagai, Yasutaka Morita, Eiichi Tamiya

School of Materials Science, Japan Advanced Institute of Science and Technology
1-1 Asahidai, Tatsunokuchi, Ishikawa, 923-1292, Japan
(yuji@jaist.ac.jp)

Abstract

This paper describes the reduction of the size of enzyme immunoassay (EIA) utilizing microchemical reaction in a microchamber array. The microchamber array was fabricated by micromachining of a silicon chip. Glass beads of 100 μm were immobilized with antibody, and put in the microchambers of 150 μm. As a competitive ELISA, sample solution mixed with HRP-conjugated antigens was allowed to react in the microchamber. As a sandwich assay, sample solution and HRP-conjugated antibody were sequentially added to the chamber. The addition of buffer, hydrogen peroxide, and fluorogenic substrate produced fluorescent dye, and a fluorescence microscope observed its fluorescence.

Keywords: fluidic self-assembly, EIA, dioxin, fluorogenic substrate, biosensor

1. Introduction

The contamination of the environment with dioxins is of concern worldwide. Dioxins are a group of persistent environmental chemicals. The term Dioxin is commonly used to refer to 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD). The name of dioxins, however, is used for the family of structurally and chemically related polychlorinated dibenzo-para-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and the coplanar polychlorinated biphenyls (co-PCBs) (Figures 1). Though some pre-concentrations are available, an analyzer for dioxins should be highly sensitive. The analysis of dioxins requires sophisticated methods that are available only in a limited number of laboratories around the world. Current methods for detection of dioxins are expensive and time consuming. An enzyme immunoassay (EIA) kit for dioxin assay has been already commercialized.

The complex nature of dioxins mixture complicates the risk evaluation for humans. The total TCDD toxic equivalents concentration (TEQs) contributed by all dioxin-like congeners in the mixture is calculated by the sum total of each congener concentration multiplied by its toxic equivalency factor. Multiple EIA, using many kinds of antibody, and the inverse operation of the functions might reveal the concentrations. The number of antibodies should be more than the number of compounds, at least 30 for the significant toxic dioxins. In order to fabricate a biosensor array that utilizes parallel antibody-reactions simultaneously, multiple immobilization of biomaterial is a key technology. Two methods for
biomaterial immobilization was developed as the progress of recent array technology, especially in DNA chip array. A photolithographic method synthesizes thousands of biomolecules on a chip combinatorially, but is applicable only to oligomer of peptide [1] or DNA [2]. A stamping method by automated robotics transfers any kind of materials. However, limited number of immobilization chemistry is available because the chemical principle in immobilization should be performed at room temperature [3].

We have proposed a new immobilization method of the biomaterial as a general-purpose method for the development of these high-integrated array-type biochips [4, 5]. Biochemical reactions using immobilized biomaterial are then performed in the microchamber. The merits of the methods are: reduced sample and reagents consumption, rapid reaction owing to reduced reaction volume, wide applicability of biomaterials without constraint, and no contamination between neighboring sites.

In this research, we employ this method to enzyme immunoassay for the detection of dioxin to reduce the size of immobilized biomaterial array. To enhance the sensitivity, fluorescence detection method was also employed.

2. Experimental

Mouse anti-dioxin antibody and its HRP conjugate were purchased from Research Diagnostics. Cross-reactivities of the antibody reported by its distributor are (2,3,7,8-TCDD 100%, 2,3,7,8-TCDF 4.6%, 2,3,7-TriCDD 20%, 2,3-DCDD 2.2%, 2-CDD 0.3%, 1,2,4-TriCDD <1.0%). Amplex Red as the fluorogenic substrate was purchased from Molecular Probes. HRP catalyzes the reaction of Amplex Red that produces resorfin (ex. 573nm, em. 590nm) (Figure 2).

A microchamber array was photolithographically fabricated on a silicon chip. Sequential treatments of the chip were washing, wet-thermal oxidation, photolithographic patterning, HF etching, anisotropic etching in TMAH, washing, and wet-thermal oxidation. The microchamber was a reversed pyramidal shape of 150 μm in one side length, and about 100 μm in depth. Glass beads of 100μm in diameter were sequentially treated with γ-APTES, glutaraldehyde, antibody, casein, and buffer solution.

As a competitive ELISA using anti-dioxin antibody, glass beads immobilized with anti-dioxin antibody were placed in the microchambers. The mixture of 1.0 ppm of HRP-conjugated 2,3,7-TriCDD and various concentrations of non-labeled 2,3,7-triCDD as sample was added to the microchambers, and allowed to react. Amplex Red containing hydrogen