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Compact quantitative optic fiber-based immunoarray biosensor for rapid detection of small analytes

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ABSTRACT

Immunoarrays have been proven to be powerful tools for high-throughput analysis of multiple analytes. In this paper, a proof-of-concept development of a novel optic fiber-based immunoarray biosensor for the detection of multiple small analytes is presented. This was developed through immobilization of two kinds of hapten conjugates, MC-LR-OVA and NB-OVA, onto the same fiber optic probe. The technique is significantly different from conventional immunoarray sensors. Microcystin-LR (MC-LR) and trinitrotoluene (TNT) could be detected simultaneously and specifically within an analysis time of about 10 min for each assay cycle. The limits of detection for MC-LR and TNT were 0.04 μg/L and 0.09 mg/L, respectively. Good regeneration performance, binding properties, and robustness of the sensor surface of the proposed immunoarray biosensor ensure the cost-effective and accurate measurement of small analytes. The change in concentration of the hapten conjugates immobilized onto the sensor surface was also proven to have no significant effection on the performance of immunoarray sensor, which is essential to the application of the immunoarray in real samples detection. This compact and portable quantitative immunoarray provides an excellent multiple assay platform for clinical and environmental samples.

1. Introduction

Analytical microarrays have emerged as powerful tools for high-throughput and rapid analysis of multiple analytes (Niessner and Seidel, 2008; Blicharz et al., 2009). Antibody and hapten arrays are specific quantitative analytical techniques using antibodies/antigens as highly specific biological recognition elements. They possess the capability to simultaneously detect numerous analytes in low sample volumes (Epstein et al., 2003; Ahn et al., 2006; Niessner and Seidel, 2008). These have been designed for a number of bioanalytical applications, such as disease diagnosis, drug discovery, proteomics assay, environmental monitoring, and detection of biological warfare agents (MacBeath, 2002; Belleville et al., 2004; Ahn et al., 2006; Kopf and Zharhary, 2007; Blicharz et al., 2009).

Despite the technological advancements made in the past decades, the successful use of microarray technology remains to be elusive for many researchers (Wingren and Borrebaeck, 2006; Kopf and Zharhary, 2007). There are many reasons for this, and a few of them are mentioned here. Conventional microarray fabrication involves spotting capture antibodies/antigens onto a heterogeneous matrix (e.g., glass slide, nylon membrane) for immobilization (Kusnezow and Hoheisel, 2002). The recognition molecules are immobilized by microprinting or microstructuring processes. Fabrication of these arrays requires expensive equipment and multiple steps, which are labor intensive and subject to some degree of variability (Wingren and Borrebaeck, 2006; Blicharz et al., 2009). Moreover, many factors influence the performance of microarray modified surfaces, including the immobilization chemistry, spotting buffer, probe concentration, and physical factors like spotter type, pins used, and environmental conditions (Ramachandran et al., 2004; Wingren and Borrebaeck, 2006). Therefore, it is essential to develop new immunoarrays which are modified by abundant bio-recognizing molecules with high activity, and have low non-specific adsorption, and good regeneration performance, and to do so without using expensive equipment and complex operation conditions.

Microarray data analysis is usually done using the complex software for image processing. It particularly presents a substantial bottleneck for many researchers (Stoeckert et al., 2002; Allison et al., 2006). Due to the inherently high variation associated with array fabrication, binding/hybridization, and image processing, the accuracy of array-based quantitative assessment is still uncertain (Clack et al., 2008; Dondrup et al., 2009). Most of microarrays cannot be regenerated, and for each multi-analyte concentration, separate microarray and positive and negative control spots are needed (Kusnezow and Hoheisel, 2002; Wingren and Borrebaeck, 2006; Allison et al., 2006).

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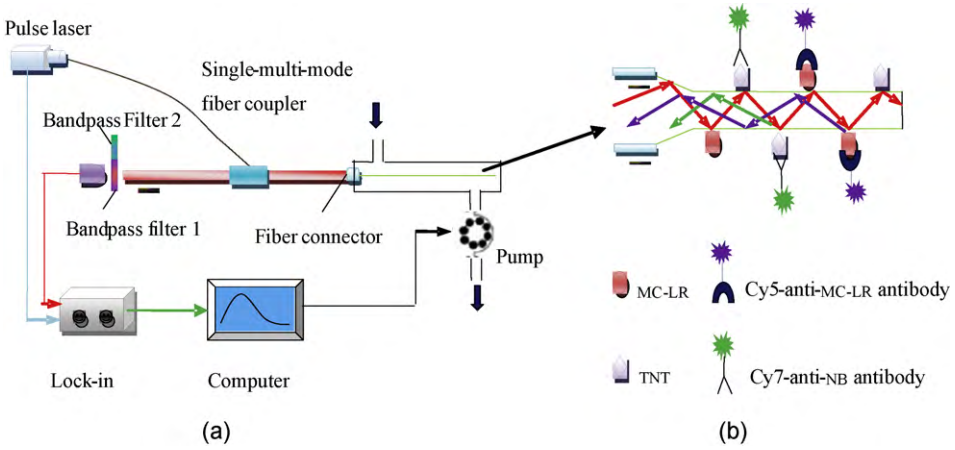

Fig. 1. (a) Schematic diagram of the structure of optic fiber-based microarray immunosensor; (b) mechanical schematic of simultaneous detection of two small analytes.

In this study, a proof-of-concept development of a compact, inexpensive, and easy-to-use optic fiber-based immunoarray biosensor is presented. This immunosensor was modified with two kinds of hapten conjugates. Its multi-analyte capabilities were demonstrated through simultaneous real-time monitoring of the binding reaction of a toxin antibody (anti-microcystin-LR or anti-MC-LR monoclonal antibody, MC8C10) and a chemistry production antibody (anti-nitrobenzene or anti-NB monoclonal antibody, NB4X10) with their counterparts (MC-LR-OVA and NB-OVA), immobilized onto the same fiber optic probe. As is already known, microcystin-LR, one of the most common microcystins, is highly toxic and contains two variable amino acids of leucin (L) and arginine (R). Many reported cases of animal poisoning and human diseases, even death, are due to MCs exposure by way of drinking and surface water (Jochimsen et al., 1998; Carmichael et al., 2001; Campàs and Marty, 2007). In this regard, the World Health Organization (WHO) has proposed an MC-LR guideline value (GV) of 1 μg/L in drinking water in order to minimize public exposure to MCs (WHO, 2004). Trinitrotoluene (TNT), another compound that is potential target for immunoassays, is a well-known explosive compound used in the preparation of landmines for military and terrorist activities (Yinon, 2002). The detection of explosives has become of tremendous importance in today's globally heightened security environment (Anderson et al., 2007). Contamination with TNT occurs through surface water runoff or ground water inflow, resulting in the accumulation of TNT in aquatic environments. Due to TNT's biological persistence, toxicity, and mutagenicity, its detection has gained considerable attention (Bromage et al., 2007). Moreover, the optic fiber-based immunoarray presented here could provide reliable quantitative results for routine application. Aside from being easy-to-use, it has faster binding kinetics, lower production cost, and regeneration and miniaturization features.

2. Experimental

2.1. Materials and reagents

Bovine serum albumin (BSA), ovalbumin (OVA), 3-mercaptopropyl-trimethoxysilane (MTS), 1-ethyl-3(-3dimethylamino-propyl) carbodiimide hydrochloride (EDC), 4-nitrohippuric acid, N-(4-maleimidobutyryloxy)succinimide, and TNT were purchased from Sigma–Aldrich (Steinheim, Germany). MC-LR was obtained from Alexis (ALX-350-012). Unless specified, all other reagents, supplied by Beijing Chemical Agents, were of analytical grade and used without further purification. Distilled deionized water was used throughout the investigation.

Monoclonal anti-MC-LR antibody (reference no. MC8C10) and anti-NB antibody (reference no. NB4X18) were produced by our research group, and labeled Cy5 and Cy7, respectively.

The hapten-carrier conjugate NB-OVA was prepared through the following processes: 101 mg 4-nitrohippuric acid was dissolved in 10 mL 0.05 M sodium phosphate-buffered saline (PBS, pH 11). Then 35 mg EDC and 20 mg OVA were added to this solution, and the reaction was incubated overnight at 4 °C under constant stirring. The NB-OVA conjugate was purified by dialysis and stored frozen at −20 °C in small aliquots until use.

MC-LR-OVA was synthesized by a modified procedure (Sheng et al., 2006). Briefly, MC-LR was treated with 2000–4000 times molar excess of 2-aminoethanethiol in a carbonate-bicarbonate buffer (pH 9.0) for 30 min at 50 °C. The product was purified from the reaction mixture on C_{18} Bond Elute cartridges with subsequent mass spectrometric identification. The resulting conjugate, H_2N-MC-LR, containing the equivalent of 1.5 mg of microcystin-LR, was then dissolved in 0.01 M PBS (pH 7.4), and mixed with 10 mg OVA. A two-step glutaraldehyde coupling procedure was carried out with 1.25% glutaraldehyde at pH 7.4. Following the coupling, the conjugate was purified by chromatography with Sephadex-G25 to ensure the absence of free toxin.

The estimated numbers of hapten molecules attached to the carrier protein (hapten-to-protein molar ratio, MR) MC-LR-OVA and NB-OVA determined by MALDI-TOF/MS were 2 and 5, respectively. 0.5 mg/mL TNT and MC-LR stock solutions were both prepared in PBS and stored at 4 °C. Standard concentrations of the analyte under analysis were prepared by serial dilutions in 0.01 M PBS from the stock solution.

2.2. Instrumentation

The schematic of the simple, compact, and portable optic fiber-based immunoarray biosensor, is shown in Fig. 1. The laser beam from a 635 nm pulse diode laser (8 mW, BWT Beijing Ltd.) with pigtail was directly launched into the single-mode fiber of the single-multi-mode fiber coupler. The laser light then entered from the single-mode fiber into the multi-mode fiber having a diameter of 600 μm and a numerical aperture of 0.22. Afterwards, the excitation light from the laser was coupled to a fiber immunosensor through the fiber connector. When the incident light propagated along the length of the immunosensor via total internal reflection, the evanescent wave was generated at the surface of the