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Multiplex detection of tumor markers with photonic suspension array

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ABSTRACT

A novel photonic suspension array was developed for multiplex immunoassay. The carries of this array were silica colloidal crystal beads (SCCBs). The codes of these carriers are the characteristic reflection peak originated from their structural periodicity, and therefore they do not suffer from fading, bleaching, quenching, and chemical instability. In addition, because no dyes or materials related with fluorescence are included, the fluorescence background of SCCBs is very low. With a sandwich format, the proposed suspension array was used for simultaneous multiplex detection of tumor markers in one test tube. The results showed that the four tumor markers, α-fetoprotein (AFP), carcinoembryonic antigen (CEA), carcinoma antigen 125 (CA 125) and carcinoma antigen 19-9 (CA 19-9) could be assayed in the ranges of 1.0–500 ng mL^{-1}, 1.0–500 ng mL^{-1}, 1.0–500 U mL^{-1} and 3.0–500 U mL^{-1} with limits of detection of 0.68 ng mL^{-1}, 0.95 ng mL^{-1}, 0.99 U mL^{-1} and 2.30 U mL^{-1} at 3σ, respectively. The proposed array showed acceptable accuracy, detection reproducibility, storage stability and the results obtained were in acceptable agreement with those from parallel single-analyte test of practical clinical sera. This technique provides a new strategy for low cost, automated, and simultaneous multiplex immunoassay.

1. Introduction

With more than 10 million new cases and 7.6 million deaths every year, cancer has become one of the most devastating diseases worldwide [1]. Yet, many of these deaths can be avoided by early detecting, treating and curing. The measurement of tumor markers has been showing its significance in early screening of cancer, differentiating benign from malignant conditions, evaluating the extent of disease, monitoring the response of cancer therapy, and predicting recurrence [2,3]. However, a single tumor marker is usually not sufficient to diagnose cancer due to its limited specificity. Thus, multiplex immunoassay of tumor markers has attracted considerable interest to meet the growing demand for diagnostic application [4–6]. Furthermore, multiplex immunoassay can offer higher sample throughput, less sample consumption, shorter assay time and lower cost than the traditional parallel single-analyte immunoassay [7].

Up to now, various assay approaches have been devised to realize simultaneous multiplex analysis. Most of them are based on molecular binding or recognition events. In order to distinguish different binding events in parallel, molecules should be encoded. Biochip, in which a large number of different probe molecules are immobilized on a flat substrate and encoded by the coordinate of their positions on a two-dimensional grid, had led to a dramatic development in the past 20 years. However, biochips still have certain drawbacks, including slow diffusion of molecules to their binding sites and the inability to perform large numbers of reactions simultaneously over a wide dynamic range [8,9]. Recently, suspension arrays, in which probes are attached to the surface of microparticles, become an attractive alternation for multiplex analysis [10–12]. Such arrays offer higher flexibility for detecting new analytes and show faster reaction kinetics in solution due to the radial diffusion of analytes or probes [13]. Among various suspension arrays, those arrays with spectrum-encoded microparticles are well used due to their simplicity in both encoding and detection. Fluorescent dyes [14–16] and quantum dots [17–20] are the main spectrum-encoding elements and the microparticles encoded by fluorescence have been commercialized by Luminex and some other companies [12]. However, the fluorescence dyes tend to be quenched or bleached and the quantum dots are usually biotoxic [21,22]. Moreover, the fluorescence of the carriers can interfere with the signal from the labeling molecules and as a result affect the detection limit. In this point of view, we proposed the silica colloidal crystals beads (SCCBs) as encoded supports for suspension array [23,24]. Code of SCCBs is the characteristic reflection peak originated from the stop-band of colloid crystal [25]. As the peak

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position is based on its periodical structure, the code is very stable and the fluorescent background is low. In addition, the SCCBs provide much higher surface-to-volume ratio which means more dye molecules participate in photon absorption and subsequent photon emission. These properties make the photonic suspension array suitable for high sensitive detection.

In this paper, we designed a photonic suspension array based on SCCBs for multiplex detection of tumor makers. The use of this array could not only increase the detection sensitivity but also simplify the decoding and bio-reaction detection process. With a sandwich format, the proposed suspension array was used for multiplex immunoassay of four tumor markers, α-fetoprotein (AFP), carcinoembryonic antigen (CEA), carcinoma antigen 125 (CA 125) and carcinoma antigen 19-9 (CA 19-9), which show great significance in early screening and clinical diagnosis of some tumor diseases including hepatocellular cancer, yolk sac cancer, colorectal cancer, gastric cancer, and lung cancer [26–28]. The results obtained were in acceptable agreement with those from the parallel single-analyte test of practical clinical sera. The photonic suspension array possesses attractive characteristics such as low cost, simple manipulation, and easy automation and has the potential to assay more analytes.

2. Experimental

2.1. Materials

Human AFP, CEA, CA125, CA19-9, and Mouse monoclonal anti-human AFP antibody, anti-human CEA antibody, anti-human CA125 antibody, anti-human CA19-9 antibody, and fluorescein isothiocyanate (FITC) tagged goat anti-human CA125 antibody, anti-human CA19-9 antibody and anti-human CEA antibody were obtained from Uscnlife Co., USA. Bovine serum albumin (BSA) was purchased from Sigma Chemicals. 3-aminopropyltriethoxysilane (APTES) and glutaraldehyde were brought from Alfa Aesar Co. Monodisperse silica nanoparticles were synthesized by Stöber method [29]. Polydimethylsiloxane (KF-96 10 cSt) was gained from Shin-Etsu Chemical, Japan. Clinical serum samples were gifts from Zhongda Hospital, China. Phosphate buffer saline (PBS, 0.05 M, pH 7.4), phosphate buffer saline tween-20 (PBST, 0.05% tween-20 in PBS) and phosphate buffer (PB, 0.05 M, pH 5.0) were self-prepared. All buffers were prepared with water purified in a Milli-Q system (Millipore, Bedford, MA). All other reagents were of the best grade available and used as received.

2.2. Instrumentation

The microfluidic device used for SCCBs generation was homemade. A needle with an inner diameter of 60 μm and outer diameter of 250 μm was inserted into the main inlet of a T-junction [24]. A magnetic pump was used to feed silica nanoparticles aqueous solution as the dispersed phase through the needle. Another inlet of the T-junction was connected to a syringe pump, through which oil used as the continuous phase was fed. The drops generated at the outlet were guided to a container for collection.

The microstructures of SCCBs were characterized by a scanning electron microscopy (SEM, HITACHI, S-300N). Photographs of SCCBs were taken with an optical microscope (OLYMPUS BX51) equipped with a CCD camera (Media Cybernetics Evolution MP 5.0). Reflection spectra of SCCBs were recorded by a microscope equipped with a fiber optic spectrometer (Ocean Optics, USB2000). Fluorescence spectra of SCCBs were recorded by a microscope equipped with a fiber optic spectrometer (Ocean Optics, QE65000).

2.3. Generation of SCCBs

The silica colloidal crystal beads were fabricated by droplet template method [24]. Firstly, the aqueous suspension containing monodisperse silica nanoparticles was broken into droplets by the oil flow in the microfluidic device, and the droplets were taken into the collection container which was also filled with the silicon oil. Then, the silica nanoparticles self-assembled into ordered lattices during the evaporation of water in the droplets at 60 °C. After solidification, the silica colloidal crystal beads were thoroughly washed with hexane to remove the silicon oil. Finally, the silica colloidal crystal beads were calcined at 700 °C for 3 h to improve their mechanical strength. To meet the demand of multiplex immunoassay, four kinds of aqueous suspension containing monodisperse silica nanoparticles with diameters of 195 nm, 247 nm, 264 nm, and 314 nm, respectively, were used for the colloidal crystal beads fabrication. The concentration (w/v) of the four kinds of silica nanoparticles was 15%. The injection speed of oil phase was 15 mL h^{-1} and the injection speed of dispersed phase was 0.5 mL h^{-1}. Finally, four kinds of 180 μm SCCBs with the reflection peak position in 428, 543, 580 and 692 nm, respectively, were generated.

2.4. Probes immobilization

Anti-tumor marker antibody probes were immobilized on SCCBs by covalent bonding method. Firstly, the SCCBs were treated with piranha solution (30% hydrogen peroxide and 70% sulfuric acid) for 6 h. After washed with water and dried by nitrogen flow, the beads were treated with an ethanol solution of APTES (5%) and PB buffer of glutaraldehyde (2.5%) for 4 h in turn. Then, the SCCBs were reacted with anti-tumor marker antibody probes (0.1 mg mL^{-1}, about 0.1 μL per bead) in PBS buffer solution at 4 °C for 12 h. Afterward, the beads were treated with 1 mg mL^{-1} $NaCNBH_3$ solution for 1 h at 4 °C. Finally, the unreacted groups of glutaraldehyde on the SCCBs' surface were passivated with 1% BSA PBS buffer for 2 h. For multiplexed immunoassays, four kinds of these SCCBs, with the reflection peak position in 428, 543, 580 and 692 nm, were modified with mouse monoclonal anti-human AFP antibody, anti-human CEA antibody, anti-human CA125 antibody, anti-human CA19-9 antibody, respectively. The fraction of immobilized antibodies was assessed by comparing the fluorescence signals produced by fluorescein isothiocyanate (FITC) tagged goat anti-tumor marker antibodies solution before and after immobilization, and the antibody immobilization efficiency was about 12%. For the immobilization of antibody, thousand of beads could be coupled per round in one tube.

2.5. Detection of tumor markers

For single analysis, different concentrations of tumor marker (tumor marker in PBS) were used to incubate anti-tumor marker antibody-modified SCCBs (1 μL per bead) in the test tubes for 30 min, and unbound tumor marker was washed away with 1% BSA PBS buffer. Then, fluorescein isothiocyanate (FITC) tagged goat anti-tumor marker antibody (10 μg mL^{-1}) was added to the test tubes and incubated for another 30 min. During all the incubation process, the test tubes were shaken at 37 °C. Fluorescence spectra of SCCBs were measured after thoroughly washing with 1% BSA PBST buffer. The number of replicates at the any concentration was 5. The detection limit was calculated from the zero calibrator plus three times the standard deviation.

For multiplexed detection of tumor markers, four kinds of SCCBs, with the reflection peak position in 428, 543, 580 and 692 nm, immobilized with anti-human AFP antibody, anti-human