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Short communication

Handy, rapid and multiplex detection of tumor markers based on encoded silica–hydrogel hybrid beads array chip

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ABSTRACT

Malignant tumor has become the leading cause of death worldwide; however, multiplex detection technology could provide great assistance in large-scale population screening of diseases which could effectively reduce the mortality of malignant tumors. Here a microbeads array chip, which could be a perfect alternative method for the early screening, was developed. Silica–hydrogel hybrid bead (SHHB) with photonic encoding, which consists of both silica and hydrogel materials, was manufactured as the carrier of microbeads array for the first time. The SHHB has the advantages of the beads made of silica or hydrogel, but does not have their limitations. Reaction conditions of SHHBs array were optimized and then the fluorescent concentration curves of two widely-used tumor markers, human alpha fetoprotein and carcinoembryonic antigen, were constructed. The accuracy of SHHBs array has been proven according to the comparison between the results obtained by detecting 50 clinical samples with SHHBs array and chemiluminescence immunoassay. A cassette like chip device has also been developed to standardize operational processes and benefit automization in the next work. Hence it is concluded that SHHBs array chip is a handy, rapid and multiplex immunoassay technology, which could imply its practical application in clinical immunoassay in the near future.

1. Introduction

Malignant tumor has become the leading cause of death all over the world. According to the report of WHO, approximately 7.6 million people, which is about 13% of all deaths, died of malignant tumor in 2008. Moreover, the number of people dying of malignant tumor may rise to more than 13.1 million in 2030 (Jemal et al., 2011). It is difficult to be cured which determines the high mortality of malignant tumor; however, early diagnosis and treatment could reduce the mortality of malignant tumors significantly (Creeden et al., 2011; Lewis and Maxwell, 2012). Both imaging and laboratory medicine could be the efficient methods for malignant tumor screening; especially laboratory medicine could screen out malignant tumors at an earlier stage (Duffy, 2012; Li et al., 2011). Tumor markers in the serum are the targets of laboratory examination, and they are the substances elevated when malignant tumors occur and develop. Tumor markers can provide great assistance in the screening, diagnosis, prognosis and drug-choice of malignant tumors (Joerger and Huober, 2012; Kelloff and Sigman, 2012). There are dozens of tumor markers used clinically, such as alpha fetoprotein (AFP), carcinoembryonic antigen (CEA), etc. Also with the assistance of proteomics, increasing numbers of new tumor markers are being discovered continuously. Correspondingly, there are various methods and matched machines developed to detect tumor markers clinically, such as chemiluminescence immunoassay (CLIA), rate nephelometry, etc. Nonetheless, these methods are usually applied in single target analysis, and require larger volume of serum samples and a longer time when the sample population is large and several targets need to be analyzed simultaneously in each sample. Therefore, multiplex immunoassay technology is necessary in high-throughput detection and it is also the direction of development of immunoassay technology in the near future. Meanwhile, some commercial analysis technologies, such as CLIA, are now approaching this objective (Yang et al., 2009, 2010; Zong et al., 2012). In this regard, microbeads array has become an ideal multiplex immunoassay technology in the last decade.

Microbeads array, also known as suspension array, is an evolutive technology of planar array, which uses microbeads with different encodings as carriers instead of position encoding as in the planar

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film. Such changes make the detection faster and more flexible with the characteristics of high-throughput, high sensitivity, and low sample consumption retained (Nolan and Sklar, 2002; Pregibon et al., 2007). Various encoding strategies of suspension array had been developed in recent years, such as fluorescent dyes encoding, shape encoding, photonic crystal encoding, etc. Among these, fluorescent dyes encoding was commercialized by Luminex and some other companies (Hsu et al., 2009). However, fluorescent dye encoding still has some problems. For instance, mutual interference between the fluorescent encoding signal and the fluorescent detection signal would exist during the decoding and the detection of results, and the fluorescent dyes are easily quenched and bleached, leading to the loss of encoding. In contrast, photonic crystal encoding is a physical encoding strategy; it uses a characteristic reflection peak or structural color as its encoding signal. Because both signals depend on the periodic arrangement structure of nanoparticles, which are basic components of a photonic crystal, the encoding signal is very stable and will not influence the detection signal. Also the reflection peak is easy to be decoded, which could benefit automization. In previous microbeads array, silica and hydrogel are the major biocompatible materials in the production of encoded microbeads owing to their excellent biological characteristics (Appleyard et al., 2011; Huang et al., 2009; Zhao et al., 2009a, 2009b). For particular photonic beads, nanosilica was the frequently-used material due to its low fluorescence background and high mechanical stability after calcination while poly ethylene glycol diacrylates (PEG-DA) hydrogel has the biological characteristics of non-toxicity, resistance of protein, etc. (Gu et al., 2008; Peppas et al., 2006; Zhao et al., 2010a). However, immunoassays based on silica photonic beads (SPBs) have excessive experimental procedures and the non-specific adsorption is difficult to clear owing to the periodic void structure of beads; the encoding of hydrogel photonic beads is unstable as the hydrogel is soft and easy to transform (Gu et al., 2008; Hu et al., 2009; Yang et al., 2012). Therefore, to solve the problems above, a novel silica–hydrogel hybrid bead (SHHB) was developed through the combination of complementary silica and hydrogel materials for the first time.

On-chip detection offers benefits in the standardization of operational processes and the development of automated testing equipment; hence it is the major direction of evolutionary clinical detection methods while increasing methods had been integrated into various chip devices (Ge et al., 2012; Han et al., 2012; Hu et al., 2010; Ikami et al., 2010; Li et al., 2005; Yang et al., 2010). SHHBs array is a sensitive and specific detection method, but it still has some minor limitations such as loss and damage of microbeads during experimental operation resulting from a small volume of microbeads, which is difficult to be distinguished with naked eyes. Hence it is necessary to integrate SHHBs array into a special chip device to remove the direct contact with microbeads. Meanwhile, it will facilitate the development of standard experiment program and suitable equipment. The main models of bioanalysis chip devices integrating microbeads array are ordered array chip, disordered cassette like chip and microfluidic chip (Han et al., 2012; Ikami et al., 2010; Zhang et al., 2010). Considering that the characters of SHHBs, such as photonic encoding and microsize, and SHHBs have to move freely during the reaction in order to have full contact with the reaction solution, a cassette like chip device was designed to integrate the SHHBs array in the study (Yang et al., 2012).

2. Materials and methods

2.1. Materials

Monodisperse silica nanoparticles used for the production of SPBs were synthesized by the Stober method (Stober et al., 1968). Human AFP and CEA standard solution, fluorescein isothiocyanate (FITC) labeled mouse monoclonal anti-human AFP and CEA antibodies were purchased from USCN Life Science, Inc., Wuhan, China. Human immunoglobulin G (IgG) standard solution and FITC-labeled mouse monoclonal anti-human IgG antibody were purchased from BioDee BioTech Co., Ltd., Beijing, China. PEG-DA with weight-average molecular weights of 700 and 2-hydroxy-2-methylpropiophenone (HMPP) photoinitiator were purchased from Sigma-Aldrich, Shanghai, China. Acrylic Acid (AA) and Tween-20 were obtained from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Aladdin Reagent Co., Ltd., Shanghai, China. Polydimethylsiloxane (PDMS) and its curing agent were purchased from Dow Corning Holding Co., Ltd., Shanghai, China. 2-Morpholinoethanesulfonic Acid (MES) was purchased from AMRESCO LLC, Solon, USA. 0.1 mol/L MES buffer (pH 6.0) was homemade. 1 × Phosphate Buffer Solution (PBS) used as protein dilution buffer and 1 × PBS containing 0.05% Tween-20 used as wash buffer were also homemade. All buffers were prepared by ultra-pure water treated in a Milli-Q system (Millipore, Bedford, USA). Clinical serum samples with the known levels of AFP and CEA detected by chemiluminescence immunoassay (CLIA) were collected from the Department Laboratory of Jiangsu Province Hospital of Traditional Chinese Medicine, China.

2.2. Instrumentation

The capillary microfluidic device used for the generation of SPBs was homemade (Shum et al., 2011; Zhao et al., 2012). Antigen–antibody reaction and washing were carried out in a constant temperature shaker (Thermomixer comfort 5355, Eppendorf, Germany). Images of SHHBs were captured by a metallographic microscope (BX51, Olympus, Japan) equipped with a CCD camera (Evolution MP 5.0, Media Cybernetics, USA). Their reflection spectra were recorded by the same microscope equipped with a fiber optic spectrometer (HR2000, Ocean Optics, USA). Microstructures of SHHBs were characterized by a scanning electron microscope (SEM) (S-300N, Hitachi, Japan). The intensity of emission fluorescence was detected by an inverted fluorescence microscope (IX51, Olympus, Japan) equipped with a fiber optic spectrometer (QE65000, Ocean Optics, USA). Image of a cassette like chip was captured by a Digital Single Lens Reflex camera (EOS 5D, Canon, Japan). Photographs of multiplex detection under bright field and dark field were captured by a stereomicroscope (MVX10, Olympus, Japan) equipped with a CCD camera (DP72, Olympus, Japan).

2.3. Pretreatment of SHHBs and reaction principle

As the precursor of SHHBs, SPBs with three characteristic reflection spectra were fabricated by capillary microfluidic device. Monodisperse silica nanoparticles are the basic component of SPBs, and their diameters determine the characteristic reflection peak positions of SPBs. After collecting in the silicone oil, drying, washing by n-hexane, calcining at high-temperature and screening, large numbers of SPBs with diameters of 250 μm ± 10 μm were produced (Shum et al., 2011; Zhao et al., 2012). Then hydrophilic treatment with piranha solution (30% hydrogen peroxide (v/v) and 70% sulfuric acid (v/v)) for 6 h was necessary. Pregel solution consists of 1% HMPP (v/v) and different proportions of PEG-DA and AA were mixed. Then SPBs were immersed into pregel solution for 30 min, by which the void of SPBs was filled with hydrogel through capillary action, and exposed to UV light for 2 or 3 min. After the hydrogel skins were peeled off, SHHBs were manufactured. Three SHHBs with characteristic reflection peaks at 491 nm, 554 nm and 688 nm were produced (Fig. S1a). After SHHBs were immersed in the mixture of MES buffer, which contains 6% EDC (w/v) and 10% NHS (w/v), and