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Photonic crystal fiber-based immunosensor for high-performance detection of alpha fetoprotein

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ABSTRACT

We have developed a sensitive photonic crystal fiber (PCF)-based immunosensor for detection of alpha fetoprotein (AFP). The unique PCF possesses a morphology characterized by numerous pore structures and a large surface area-to-volume ratio, which can be used as an immune-reaction carrier to improve the sensitivity and reaction speed of AFP detection. The PCF-based immunosensor possesses a low limit of detection of 0.1 ng/mL, which is five times lower than that of the capillary-based sensor and 35 times lower than that of the traditional enzyme-linked immunosorbent assay. The wide linear dynamic range of 0.1–150 ng/mL makes the developed immunosensor suitable for clinical practice. The proposed method was successfully applied to AFP detection in a clinical serum sample with acceptable precision. It is indicated that the present PCF-based immunosensor could be used as an attractive analytical platform for sensitive and specific detection of cancer biomarkers.

1. Introduction

Given the highly specific molecular recognition of the antibody and epitopes of an antigen, immunoassay techniques have become important tools in quantitative clinical detection of cancer biomarkers (Wu et al., 2007; Yin et al., 2010). Traditional immunoassays on a 96-well microtiter plate, such as enzyme-linked immunosorbent assay (ELISA), typically involve relatively large amounts of reagents, time-consuming, and tedious procedures. In addition, the sensitivity of traditional ELISA is inadequate to detect trace targets in complicated samples, and the linear range is not sufficiently wide to detect some parts of cancer markers. Thus, it is necessary and important to develop a sensitive method with broaden linear range for detection of cancer biomarkers in real clinical samples.

Miniaturized immunoassay devices or immunosensors, which combine the high specificity of the immunological reaction with sensitivity and convenient operation, have been developed rapidly in recent years (Chen et al., 2016; Gopinath et al., 2014; He et al., 2016; Lee-Lewandrowski and Olivo, 2013; Liu et al., 2015; Sun et al., 2014). A widely used approach to construct immunosensors is the application of capillary tubes (Hu et al., 2013; Mastichiadis et al., 2008, 2009; Moser et al., 2014; Niotis et al., 2010; Yang et al., 2014; Yu et al., 2014, 2013; Zhang et al., 2015), which present several advantages over other types of solid supports. For example, capillary-based immunoassays are fast, sensitive, and easy to automate, and capillary walls can act as light waveguides of excitation or emission light and fluid cells. Despite these technical advances, capillary-based immunosensors remain commercially unavailable because improvements in detection sensitivity and reliability are still required. A particular shortcoming of a capillary-mediated immunosensor is the surface expansion of the capillary tube, which is inherently limited by the 1D cylindrical geometry. Duan et al. recently proposed a 3D capillary-based immunosensor for detection of carcinoembryonic antigen (CEA), which was fabricated by placing eight capture antibody-coated capillary tubes inside a quartz tube with epoxy on both ends and by fluorescence collection with aluminum foil to reflect the laser beam (Yu et al., 2014). Despite the complicated construction and flow system setup of the immunosensor, the designed sensor increased the area of the sensing surface and resulted in an analytical performance improvement of 3.4-fold. To overcome the disadvantage of capillary tube, photonic crystal fiber (PCF), a new class of optical fibers, has been regarded as analogous to a bundle of capillary tubes with a much narrower diameter of only several micrometers. PCF is characterized by a periodic arrangement of air holes running along the entire length of the fiber (Russell, 2003). Since

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its development by Russell in 1996, PCF has been widely applied in fiber-optic communications, fiber lasers, nonlinear devices, high-power transmission, and other areas because of the properties of the photonic crystal (Cubillas et al., 2013; Jin et al., 2013; Villatoro and Zubia, 2016). Emerging but still scanty applications of PCF include fabrication of microreactors and biosensors, which has already demonstrated advantages in the improvement of analysis sensitivity. In most applications, PCF is utilized as a container of the sample and a light guide for probing; thus, fluids and probing electromagnetic waves enter and exit at the end facets of the fiber (Padmanabhan et al., 2010; Ruan et al., 2007). A sophisticated system setup is required to couple the surrounding optical and fluidic infrastructure, and caution should be applied to maintain a stable optical alignment, especially when splicing conventional single-mode fiber into large-hole small-core PCF (Cardenas-Sevilla et al., 2013; Rindorf et al., 2006). Compared with capillary tubes, the advantage of PCF is large surface area-to-volume ratio, thus, PCF is an attractive carrier in immune-reaction to enhance the sensitivity of immunoassay.

Here, we have developed a new PCF-based immunosensor by utilizing the high-sensitive laser-induced fluorescence (LIF) detection system and commercial-available PCF as the support to immobilize antibody in the immune- reaction. Fig. 1 shows schematically the construction of our PCF-based immunosensor. The unique PCF geometry results in large area for molecule recognition and immobilization, which is beneficial to sensitivity. The optical setup of the proposed PCF-based immunosensor is simplified by using PCF as the immunoassay vessel and LIF detection at the surface. Our study shows that PCF presents the potential to be used as a general sensor component that is robust and easy to package in various assay devices. The significant advantages of PCF over capillary tubes include remarkably increased surface-to-volume ratio, reduced sample volume, and natural 3D geometry. Furthermore, mass transport is highly efficient for narrow holes (several μm in diameter) because of the small diffusion distances. This feature can shorten the entire analysis time. Therefore, the application of PCF is expected to result in improved performance relative to the use of capillary-based immunosensor.

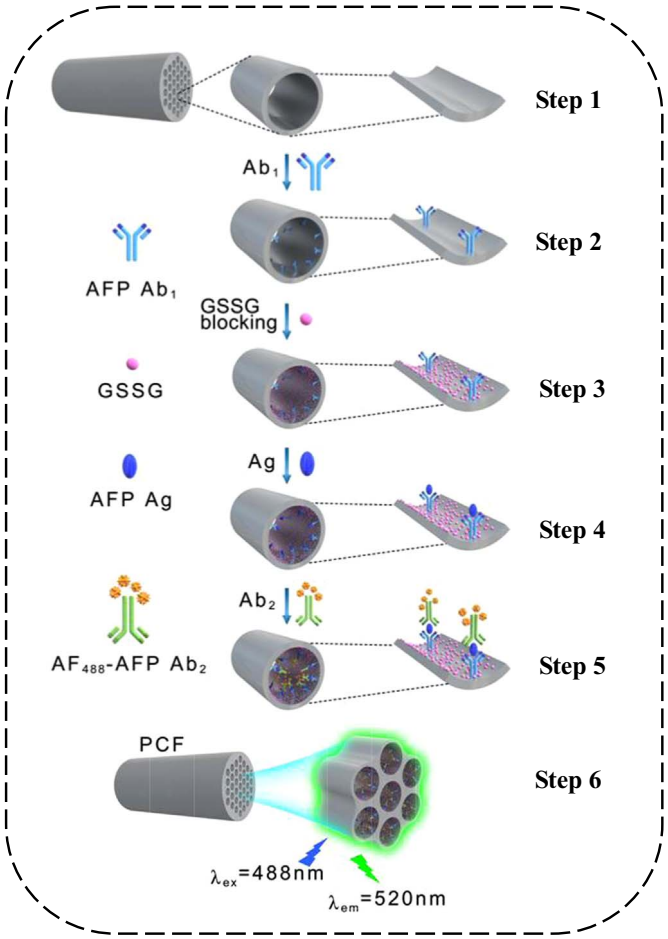

Fig. 1. Schematic diagram of the PCF-based immunosensor for the detection of AFP.

To illustrate its functionality we have applied the PCF-based immunosensor for detection of alpha fetoprotein (AFP) in human serum. AFP is an oncofetal glycoprotein with a molecular mass of approximately 70 kDa, and is an important clinical tumor marker for hepatocellular carcinoma (Fan et al., 2014; Li et al., 2014). AFP is mainly produced by the liver, yolk sac, and gastrointestinal tract of a human fetus (Ahn et al., 2015; Ji et al., 2015). In healthy human serum, the average concentration of AFP is typically less than 25 ng/mL (Tamura et al., 2009). The increased AFP concentration in adult plasma is usually considered as an early indication of hepatocellular carcinoma or endodermal sinus tumor. Our study shows that the present PCF-based immunosensor is an efficient approach for sensitive detection of AFP in real serum samples, which is of great importance to early diagnosis and treatment of hepatocellular carcinoma.

2. Material and methods

2.1. Reagents and materials

PCF (LMA-20, 20-μm core diameter, 4–5-μm average diameter of the voids) was purchased from NKT Photonics A/S (Denmark). Fused-silica capillary (50 μm i.d, 365 μm o.d) was purchased from Yongnian Optical Fiber Factory (Hebei, China). The detection and capture monoclonal antibody pair, Alexa Fluor 488 (AF_{488})-labeled mouse antihuman AFP antibody and mouse antihuman AFP antibody, and AFP human antigen were obtained from Beijing Bioss Biotech (Beijing, China). Oxidized glutathione (GSSG) was purchased from Sigma Chemical (St. Louis, MO) and was used as the blocking solution. 3-Aminopropyl diethoxymethylsilane (3-ADMS), glutaraldehyde (GA) and the interfering agents (ascorbic acid, glucose, leucine, glycine, and glutamic acid) were purchased from Sigma Chemical (St. Louis, MO). CEA and IgG were supplied by National Center for Nanoscience and Technology. PBS buffer was employed as a wash buffer and was sterilized and prepared fresh daily.

AFP real samples (serum samples) were collected from Beijing Friendship Hospital (Beijing, China) in accordance with the rules of the local ethical committee. The serum samples were diluted with PBS buffer by 10 folds for analysis (n=3).

2.2. LIF signal readout system

A 488-nm output of a diode laser (Cobolt AB, Sweden) was used as the excitation light source in LIF detection. The laser power was set at 1 mW to suppress the influence of fluorescence bleaching. The laser beam passed through a 488-nm band-pass filter and focused by a focal-length fused-silica planoconvex lens (23-mm diameter) onto the center of the PCF. The fluorescent photons emitted from a sample in the PCF were collected at 90° from the exciting light by a microscope objective and filtered using a 520-nm band-pass filter, and finally measured using a CH-253 photomultiplier tube (PMT, Beijing Hamamatsu Photon Technique INC) operated at 800 V. The PMT was powered with a home-built power supply. The PCF was mounted in home-built holder. Data acquisition was accomplished with a chromatography work station (Vanshine Instrument CO., LTD. Shanghai, China).

2.3. The process of PCF-based immunosensor

As shown in Fig. 1, the immunoassay process using the PCF-based sensors involves the following six steps: 1) The surface of PCF is pre-treated with 3- ADMS and GA, leaving an aldehyde-activated surface.