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Antibody modified gold nano-mushroom arrays for rapid detection of alpha-fetoprotein

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ABSTRACT

Localized surface plasmon resonance (LSPR) combined with immunoassay shows greatly potential in fast detection of tumor markers. In this paper, a highly sensitive LSPR substrate has been fabricated and modified for direct detection of alpha-fetoprotein (AFP). The biosensor was prepared by interference lithography, and modified by covalently immobilizing anti-AFP on the surface of gold nano-mushroom arrays (GNMA). The modification process was investigated by Vis–NIR reflectance spectra and cyclic voltammogram measurements. We revealed the optical properties of the modified GNMA by measuring the Vis–NIR reflectance spectra and simulating its electric intensity field distribution under light illumination. The GNMA substrate was highly sensitive, with a refractive index sensitivity of ~465 nm/RIU. The substrate can be applied to label-free detection of AFP, with the linear range and the limit of detection determined to be 20–200 ng/mL and 24 ng/mL ($S/N=3$), respectively. We also demonstrated its clinical application by directly detecting AFP in human serum samples. It is expected that our biosensor could be integrated on microfluidic chips for high-throughput detection in portable early diagnosis, post-operative and point-of-care (POC) in clinical applications.

1. Introduction

Rapid detection is of importance in many areas such as clinical diagnosis (Boehme et al., 2010), food safety (Velusamy et al., 2010) and environment monitoring (Girones et al., 2010). Especially in mobile healthcare (Free et al., 2013), the need for fast detection of trace sample is a key issue to early diagnosis, point-of-care, portable medical supervision, and antibody, as well as biomarker detection/screening for many serious diseases. For example, cancer, accounting for 8.2 million deaths among 14 million cases in 2012, for which there is neither a cure nor a good clinical diagnostic, has attracted world-wide attention. A cancer biomarker (*e.g.* alpha-fetoprotein, AFP) is a 'molecular signature' of the physiological state of a cancer at a specific time, because the present at elevated or depressed concentrations of cancer biomarker in serum, tissue, or saliva can be indicative of disease states. The availability of rapid detection of tumor biomarkers (TMs) is significantly useful to patients suffered from cancer, initiating both the effective treatment at an as-earlier stage in clinical and the long-term point of care for post-operative evaluation during hospitalization and after discharge. Therefore, the reliable, cost-effective, powerful methods for cancer biomarker detection and monitoring are extremely important.

A variety of methods have been developed for the detection of TMs, such as electrochemiluminescent/chemiluminescent immunoassay (Liang et al., 2012; Wang et al., 2012), enzyme-linked immune sorbent assay (Ju et al., 1999; Nagasaki et al., 2007), fluorescent immunoassay (Christopoulos and Diamandis, 1992; Yuan et al., 2001), electrochemical immunoassay (Su et al., 2011; Zhang et al., 2008), and surface plasmon resonance (SPR) immunoassay (Scarano et al., 2010; Suwansa-ard et al., 2009; Teramura and Iwata, 2007), *etc*. Despite the employ of quality and reliable reagents, these methods are confronted with one or more shortcomings with respect to sensitivity, detection velocity, and configure simplicity against rapid analysis of TMs: (1) The sensing substrates have been previously bio-functionalized to increase the specificity, which, unfortunately, bring about the undesirable consequence (*e.g.* greatly lowing the sensitivities) into some immunoassay, such as EC and SPR immunoassay; it becomes a critical factor for the detection of trace analytes with concentration range from pM to fM (Estevez et al., 2014). (2) Many strategies (Christopoulos and Diamandis, 1992; Ju et al., 1999; Liang et al., 2012; Nagasaki et al., 2007; Su et al., 2011; Wang et al., 2012; Yuan et al., 2001; Zhang et al., 2008) tend to employ a sandwich structure

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where labeled secondary recognition elements are added for generation and/or amplification signals. However, these measures inevitably complicated the detection system and lengthened total analysis time. (3) Though SPR holds the advantages of label-free and real-time (Estevez et al., 2014; Scarano et al., 2010; Suwansaard et al., 2009; Teramura and Iwata, 2007) over the other methods, its optical path configure is complex and uneconomic because a prism or a grate must be used to overcome the momentum mismatch, so that the SPR can be readily excited. Therefore, a simple and highly-sensitive method for rapid detection of TMs on a portable device is still a challenge.

Recently, localized surface plasmon resonance (LSPR) nanosensors enjoy a reputation for powerful quantitative assay (Anker et al., 2008; Haes et al., 2005; Yang et al., 2014; Zhou et al., 2011), as they are simple, cost-effective and suitable for detecting proteins. Many efforts have been made to improve the sensing performance of the LSPR sensor by changing the size and shape (Lee and El-Sayed, 2006; Nehl et al., 2006), and/or the spatial arrangement (Atay et al., 2004; Hatab et al., 2010) of the noble metal nanoparticles and nanostructure. In our previous work, we have demonstrated that gold nano-mushroom arrays (GNMA) substrate possesses excellent refractive index (RI) sensitivity (Shen et al., 2013) approaching the theoretical limit. The GNMA substrate therefore is a promising candidate as a RI nanosensor for rapid detection of TMs. For the specific immunoassay, the metal surface of LSPR sensors commonly should be functionalized with antibody, and their plasmonic optical properties therefore would be tuned (Min et al., 2009). To the best of our knowledge, the LSPR feature of the functionalized sensor, indeed, characterizing the authentic immunoassay performance, has been barely investigated yet.

Herein, we prepared an antibody-functionalized GNMA nanosensor with high sensitivity, and applied it to proof-of-concept direct detection of AFP. The GNMA substrates were fabricated as described before. As showed in Scheme 1, the GNMA substrate was covalently functionalized with AFP antibody. We investigated the authentic LSPR sensing performance of the modified GNMA substrate by investigating its LSPR feature, and found that it was highly sensitive and quick-response to RI changes of the adjacent environment. After that, we applied the GNMA biosensor to label-free detection of AFP with ultralow concentration, and obtained a limit of detection (LOD) estimated to be 24 ng/mL. Furthermore, we demonstrated its clinical application by directly detecting AFP in human serum samples. We believe the anti-AFP functionalized GNMA substrate is available to rapid detection of AFP in mobile healthcare.

2. Materials and methods

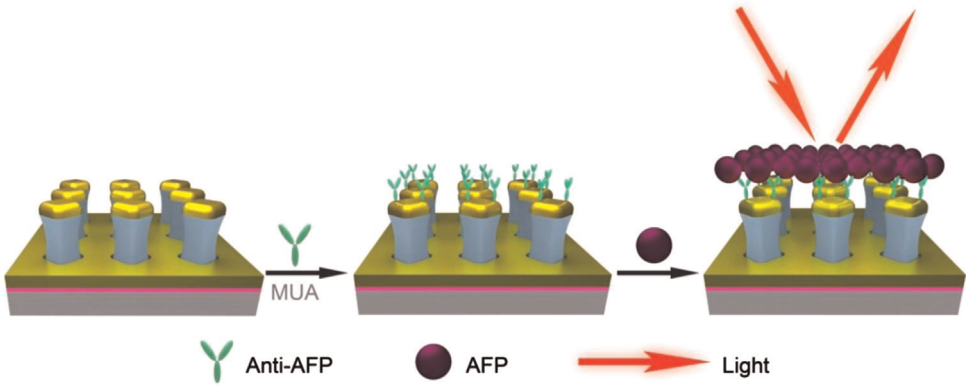
2.1. Materials

11-Mercaptoundecanoic acid (MUA) and 3-mercaptopropionic acid (MPA) were from Alfa Aesar (US). 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) was purchased from Fluka Analytical (US). N-Hydroxysuccinimide (NHS) and 6-mercapto-1-hexanol (MCH) were supplied by Sigma Aldrich (US). NaH_2PO_4 and Na_2HPO_4 were from Da-Mao chemical reagent (Tianjin, China). All chemicals and solvents were of analytical grade and were used as received. Phosphate buffer solutions (PBS, 0.1 M) were prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4, and then adjusting the pH to 7.2. AFP monoclonal antibody and AFP standards were all from Biocell Biotechnology (Zhengzhou, China) and stored at 4 °C as received. Double distilled water was used throughout all experiments.

2.2. Fabrication of the GNMA biosensor

The GNMA biosensor was prepared by antibody-functionalizing the GNMA substrate. The GNMA substrate was generated through interference lithography (IL) and then gold deposition (Shen et al., 2013). In brief, a thin positive photoresist (AZ 3740, Allresist) film (~500 nm thickness) was spin-coated on a quartz slide, baked, and then applied to IL to generate a periodical pillars array. The GNMA was obtained after sequential thermal evaporation of 5-nm nickel and 110-nm gold on the photoresist pillars array.

The GNMA substrate was antibody-functionalized by covalently immobilizing anti-AFP onto the thiolated modified gold surface of the substrate. The gold surface was firstly thiolated with MUA (Wilbur et al., 1996). A MUA solution (20 mM) was applied to a flat PDMS; the PDMS slab was dried in a stream of nitrogen for ~1 min and brought into contact with the surface of gold substrate. After ~30 s, the thiol molecules self-assembled onto the gold surface rapidly through covalent bonding to form a monolayer, and the PDMS slab and gold substrate were carefully separated. After that, the GNMA-MUA surface was covered by MPA solution (2 mM) for ~12 h. These treatments ensured that the gold surface of the GNMA substrate was covered by a self-assembly monolayer (SAM) of molecules containing pendent –COOH groups via covalent thiol chemistry. The un-reacted MUA and MPA molecules were washed away by PBS and deionized water. After thiolation, AFP antibody was covalently bonded to the –COOH groups. The –COOH groups were further activated by exposing them to 20 μL of freshly prepared EDC (400 mM) and NHS (100 mM) mixture solution, and the reaction solution was washed off after ~30 min; this was immediately followed by 2-h



Scheme 1. Schematic diagram shows that the GNMA substrate was functionalized with anti-AFP, and then was applied to one-step detection of AFP. The reflectance signal was supervised by UV–vis–NIR spectrometer under 30° incidence wave.