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Sensitive detection of multiple mycotoxins by SPRi with gold nanoparticles as signal amplification tags

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ABSTRACT

Detection of multiple toxic mycotoxins is of importance in food quality control. Surface plasmon resonance imaging (SPRi) is an advanced tool for simultaneously multiple detections with accuracy; however, it suffers from limited sensitivity due to the instrumental constraint and small sizes of mycotoxins with only one epitope for an insensitive competitive immunoassay. In this work a gold nanoparticle (AuNP)-enhanced SPRi chip is designed to sensitively detect multiple mycotoxins using a competitive immunoassay format. The sensing surface is constructed by uniformly attaching dense mycotoxin antigens on poly[oligo(ethylene glycol) methacrylate-*co*-glycidyl methacrylate] (POEGMA-*co*-GMA) brush modified SPRi gold chip. After competitive binding in a sample solution containing respective monoclonal antibodies, secondary antibody-conjugated AuNPs are employed to bind with the captured monoclonal antibodies for further amplification of the SPRi signal. Highly specific and sensitive simultaneous detection is achieved for three typical mycotoxins including Aflatoxin B1 (AFB1), Ochratoxin A (OTA) and Zearalenone (ZEN) with low detection limits of 8, 30 and 15 pg mL^{-1} and dynamic ranges covering three orders of magnitude.

1. Introduction

Mycotoxins are a group of secondary metabolites produced by fungi including *Aspergillus* and *Fusarium*. They widely exist in a large variety of agricultural products, foodstuffs, feeds and environment and are dangerous contaminations with strong toxicity, carcinogenicity, teratogenicity, and mutagenicity [1–5]. Most mycotoxins are heat-stable molecules and easy to remain and accumulate in the food chains, which further brings threats to the health of human and animals [6]. Strict regulations for the allowed levels of various mycotoxins in food products have been set by official food regulation authorities in different countries. Considering the high danger and wide existence of these mycotoxins, it is important to quantitatively detect mycotoxins for food safety. Current analysis of mycotoxins mainly relies on chromatography techniques such as high-performance liquid chromatography (HPLC), which is laborious and time intensive, and requires expensive equipments and skilled personnel [7–9]. Alternatively, immunoassay-based techniques such as enzyme-linked immunosorbent assay (ELISA) and electrochemical/optical immunosensors have been employed for mycotoxin detection by using the specific affinity between a mycotoxin with its antibody [10–13]. Immunoassay-based techniques allow for quantification of mycotoxins with good reliability and selectivity, but it remains a great challenge to simultaneously detect multiple mycotoxins with high sensitivity and excellent specificity.

High-throughput detection technologies have sparkled intensive interest for simultaneous detection of multiple targets of interest [14–17]. Fluorescence microarray is one of the choices for high-throughput detection by spotting multiple immunoassay probes (antibody, antigen, or aptamer, etc.) on a substrate in a spatially addressable manner and monitoring parallel biomolecular interactions using fluorescence-labeled signal-reporting tags [3,18,19]. However, fluorescence microarray depends on the

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fluorescence-labeled antibody for signal readout, which elevates the detection expense and assay duration. Also, fluorescence is susceptible to photo-bleaching and fluorescent labels may damage the recognition activity of the biomolecules, both of which may induce uncertainty of the immunoassay.

Compared with fluorescence, Surface plasmon resonance imaging (SPRi) is another valuable signal transducer for microarray immunoassay with the advantages of label-free and real-time [20–23]. It is able to track parallel affinity reactions between non-labeled biomolecules by monitoring the *p*-polarized light induced Surface plasmon resonance (SPR) properties occurring on a gold thin film, thus allowing for high-throughput detection and providing kinetic information on the affinity binding between native biomolecules [21,24–26]. However, it is a great challenge using SPRi for sensitive detection of mycotoxins as the mycotoxins are small molecules, and their bindings with their antibodies are hard to arouse detectable SPRi signals. With competitive immunoassay format, mycotoxins could be quantified, but low signal-to-noise ratio and poor sensitivity often greatly hurdle the practical application. Thus reliable signal amplification of SPRi is highly demanded for specific and sensitive detection of mycotoxins.

Utilizing specific reaction/bioconjugate to amplify the weak SPRi signal originated from biomolecular interaction is an efficient way toward sensitive SPRi immunoassay as the reaction products and/or bioconjugates introduced via biological affinity interaction largely enhance the dielectric constant change near the sensing surface for improved immunoassay performance [27–32]. In this regard, biofunctionalized nanomaterials such as gold nanoparticles (AuNPs), magnetic nanoparticle (MNPs), and quantum dots (QDs) have been employed as amplification tags [29–32]. Enzyme-catalyzed precipitation and atom transfer radical polymerization (ATRP) have also been developed for sensitive immunoassay [27,28]. However, it remains a great challenge to reliably amplify the SPRi signal for improved sensitivity while retaining the specificity of the immunoassay, especially for the small molecule detection with competitive format.

In this paper we report a sensitive SPRi chip for simultaneous detection of multiple mycotoxins by using antibody-conjugated AuNPs to intensify the signals on a poly[oligo(ethylene glycol) methacrylate-*co*-glycidyl methacrylate] (POEGMA-*co*-GMA) brush modified SPRi chip. We chose AuNPs as signal amplification tags because they are able to efficiently enhance the SPRi signal and particularly, their diameters could be well controlled with narrow size distribution by well-established synthetic methods and could be reliably conjugated to antibodies with good suspension stability and entire retention of the recognition capacity of antibodies, thus allowing efficient signal enhancement with low variations [33,34]. Meanwhile, the POEGMA-*co*-GMA brush, possessing high while uniform antigen loading capacity and excellent resistance to non-specific protein adsorption, is employed as a surface modification layer to minimize the spot-to-spot variation and to improve the signal-to-noise ratio [35–37]. With as-reported SPRi chip, three typical mycotoxins including Aflatoxin B1 (AFB1), Ochratoxin A (OTA) and Zearalenone (ZEN) (chemical structures are shown in Fig. 1) are detected, showing low detection limits, excellent specificity and wide dynamic ranges covering three orders of magnitude.

2. Experimental

2.1. Fabrication of SPRi chip

POEGMA-*co*-GMA was firstly grown on the SPRi gold chip with a surface-initiated atom transfer radical polymerization (SI-ATRP) [21]. In detail, a clean SpotReady™ SPRi chip was firstly dipped into a 1 mg mL^{-1} Cysteamine ethanol solution for 24 h incubation, followed by intensive rinsing with ethanol. After drying with gentle nitrogen flow, this Cysteamine monolayer modified chip was immersed in a 10 mL tetrahydrofuran (THF) solution containing 77 μL triethylamine (TEA) and 64 μL 2-bromoisobutyryl Bromide (BIB) in ice bath for 2 h incubation, followed by rinsing and drying. The initiator-attached chip was further dipped into a 25 mL SI-ATRP growth solution in a 50 mL centrifuge tube, which contains 1:1 (v/v) H_2O/methanol solution with 10% (v/v) oligo(ethylene glycol) methacrylate (OEGMA, Mn = 360), 0.5% (v/v) glycidyl methacrylate (GMA) as monomers and 2.3 mg mL^{-1} 2,2′-bipyridyl (Bipy), and 1.68 mg mL^{-1} copper (II) bromide ($CuBr_2$) as the ATRP catalyst. The SI-ATRP is triggered by rapidly adding 65 mg ascorbic acid predissolved in 1 mL deionized H_2O to the solution with strong stirring. After sealed and kept in inert atmosphere for 6 h, the chip was rinsed with ethanol and H_2O, respectively and dried under gentle nitrogen flow.

Antigen chip was prepared by manually dropping ca. 0.3 μL antigen solution (AFB1-BSA, OTA-BSA, ZEN-BSA, or BSA as control, 200 μg mL^{-1} in 0.01 M PBS) onto each spot of the POEGMA-*co*-GMA modified pre-patterned chip. The gold chip with antigen solution was placed in a dry cabinet at room temperature for 12 h, followed by intensive rinsing with 0.01 M PBS and drying with gentle nitrogen flow for SPRi measurement.

2.2. Production of monoclonal antibodies

Monoclonal antibodies including anti-AFB1, anti-ZEN and anti-OTA were produced with the hybridoma antibody technology. In detail, antigen (mycotoxin conjugated BSA) was multiple-site subcutaneously injected into Balb/c mouse separately and four immunizations were preformed to select the hybridomas of high sensitivity and good specificity with a two-step ELISA screening procedure. The hybridoma cells selected were then intraperitoneally injected into Freund's incomplete adjuvants (FIA) treated Balb/c mice to prepare ascites for production of monoclonal antibodies.

2.3. Synthesis of antibody-conjugated AuNPs

Antibody-AuNP conjugate was prepared by dropwise adding 0.5 mL AuNP solution synthesized by citrate reduction method into 1 mL of 100 μg mL^{-1} polyclonal antibody (anti-Mouse IgG) solution in 0.01 M PBS with gentle stirring [34]. The mixed solution

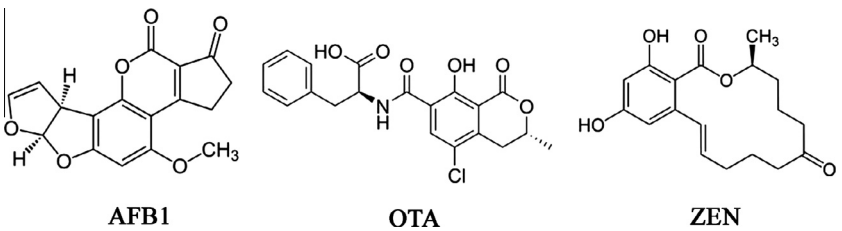

Fig. 1. Molecular structures of AFB1, OTA and ZEN.