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Molecularly imprinted polymer grafted paper-based multi-disk micro-disk plate for chemiluminescence detection of pesticide

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ABSTRACT

The detection of pesticides has attracted considerable attention in numerous fields, such as environmental monitoring and food safety. Although traditional sensors for pesticides have been widely explored due to their high sensitivity and specificity, it is still challenging to develop a low-cost, portable, fast, and easy-to-use detection method for the public use at home or in the field. To address these challenges, herein, we report a novel paper-based molecular imprinted polymer (MIP)-grafted multi-disk micro-disk plate (P-MIP-MMP) for sensitive and specific chemiluminescence (CL) detection of pesticides through an indirect competitive assay using 2,4-dichlorophenoxyacetic acid (2,4-D) as a proof-of-concept analyte. The MIP-grafted paper disks were prepared by a simple in situ polymerization of MIP layer on the surface of cellulose fibers in paper. The quantification mechanism of this P-MIP-MMP is based on a competition between free 2,4-D and tobacco peroxidase (TOP) labeled 2,4-D and the enzyme catalyzed CL emission from the luminol-TOP-H_2O_2 CL system. At optimal conditions, this P-MIP-MMP can detect 2,4-D at the concentration of femtomolar level. This approach provided a powerful protocol for simple, low-cost, rapid, and high-throughput detection of pesticides in real samples with satisfactory results for use in areas such as food inspection and environmental monitoring.

1. Introduction

Pesticides contribute significantly in maintaining food quality and production. But simultaneously they have a tremendous negative effect on natural life and human health. Approximately 800 pesticides are currently in use. One of the pesticide that has attracted most research interest and that was used as a model pesticide in many investigations is 2,4-dichlorophenoxyacetic acid (2,4-D), which is an endocrine disruptor commonly used to control broadleaf weeds. It belongs to the top 10 hit-list of pesticides used all over the world (Barzen et al., 2002), as it may lead to cancer in humans (Ibrahim et al., 1991), and endocrine-disrupting activities (Burns et al., 2001). Therefore, development of highly selective and sensitive analytical methods for 2,4-D detection in the environment is of great significance. For the past years, a number of well-established analytical methods such as gas chromatography (Ranz et al., 2008) and high performance liquid chromatography (Shin et al., 2011) methods have been reported for the detection of 2,4-D at the trace levels. However, these methods were complex, time-consuming, and require costly and bulky instrumentation. Many researchers now employ immunoassay, the prevailing method for detecting pesticides, for the detection of 2,4-D with different sensing methodologies (Halámek et al., 2001; Long et al., 2012; Švitel et al., 2000).

Although these immunoassays have their own advantages, low-cost, simple, and high-throughput methods are still needed to screen the specific target pesticide rapidly for the food inspection and environmental monitoring in extremely resource limited locations. The recently developed microfluidic paper-based analytical devices (μ-PADs), which use hydrophilic paper as a platform for multiplexed analyte detection, have emerged as a promising solution to the need for low-cost and simple analytical systems (Martinez et al., 2010; Sia and Kricka, 2008; Whitesides, 2011). Paper, a three-dimensional (3D) cellulose fiber web with a high surface area, represented a great supporting material for biosensing devices due to its demonstrated advantages (Pelton, 2009): First, it is abundant, inexpensive, biodegradable, disposable, and easy to use, store, and transport. Second, its porous cellulose fiber network acts as capillaries, wicking aqueous solutions without the need for active pumping. The μ-PADs could be extremely useful as outstanding analytical platforms in remote regions or developing countries where simple, portable, disposable, and inexpensive bioassays are essential in the first stages of disease detecting, environmental monitoring, and food inspection in the field.

In recent years, the developments of immunoassay on μ-PADs, such as colorimetric immunoassay (Apilux et al., 2012; Cheng et al., 2010; Fu et al., 2011; López_Marzo et al., 2013; Oh et al., 2013), fluorescent immunoassay (Liang et al., 2012; Miranda et al., 2013),

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chemluminescent (CL) immunoassay (Ge et al., 2012a; Wang et al., 2012c; Wang et al., 2012d), electrochemical immunoassay (Ge et al., 2012c; Wang et al., 2012a; Wu et al., 2012; Zang et al., 2012), electro-generated CL immunoassay (Ge et al., 2012b; Wang et al., 2012b; Wang et al., 2012e; Yan et al., 2012), localized surface plasmon resonance immunoassay (Tian et al., 2012), and photoelectrochemical immunoassay (Ge et al., 2013a; Wang et al., 2013a), are gaining importance because of their low-cost, simple, and high-throughput screening capabilities. As a facile, fast, sensitive, and cost-effective analytical technique, CL method has been proven a powerful analytical tool for μ-PADs (Ge et al., 2012a; Wang et al., 2012c; Wang et al., 2012d; Yu et al., 2011a; Yu et al., 2011b), due to the unemployment of the excitation light source and the operational simplification of the equipment as well as its high sensitivity and wide dynamic range.

However, while these immunoassays on μ-PADs showed promise, all of them relied on antibodies. Nevertheless, these biological recognition elements have some fundamental drawbacks, for instance, possible denaturation and instability during manufacture and transportation. Molecular imprinting, originally developed in the 1970s (Wulff, 1995), is an appropriate technique for the preparation of bio-mimetic receptors for target molecules (Haupt and Mosbach, 2000; Priego-Capote et al., 2008). The target molecule acts as a template around which interacting and cross-linking monomers are arranged and polymerized to form a cast-like shell. Following template removal, complementary cavities with memory of the shape, size, and functional groups of the template molecules, are formed in the highly cross-linked polymer matrix, which can rebind the template molecules from a mixture of closely related compounds with very high specificity, comparable to that of natural receptors. It has been suggested that molecularly imprinted polymers (MIPs) could provide an alternative to antibodies for use as recognition elements on μ-PADs in our previous work (Ge et al., 2013b), owing to their high chemical and physical stability, ease of preparation, and low price (Haupt, 2003). The MIP layer grafted on the surfaces of cellulose fibers in paper showed enhanced surface area and shortened rebinding time (Ge et al., 2013b) mainly due to the high surface-to-weight ratio and 3D incompact porous structure of paper. The shortened rebinding time could be favorable to high sample throughput and rapid application. To the best of our knowledge, no report about establishing MIP-based CL detection on μ-PADs has been published.

Due to the increased applications of pesticides in agriculture as well as the increased attentions in food safety and environment control, the number of samples to be tested increased remarkably. Thus, there is a great demand for high-throughput assays of pesticides. Recently, paper-based multi-zone micro-zone plates (Alkasir et al., 2012; Carrilho et al., 2009; Cheng et al., 2010; Jokerst et al., 2012; Veigas et al., 2012; Wang et al., 2012c; Wang et al., 2012d) are frequently used, and they have become the basic testing devices for high-throughput, simultaneous, and replicate assays. A unique advantage of paper-based multi-zone micro-zone plates is that they can test a large sample set using colorimetric, CL, fluorescent, or electrochemical detecting techniques and obtain rapid semi-quantitative or quantitative results via either visual detection (Alkasir et al., 2012; Jokerst et al., 2012; Veigas et al., 2012) or simple instrumental detection such as plate reader (Carrilho et al., 2009; Cheng et al., 2010; Wang et al., 2012c; Wang et al., 2012d; Wang et al., 2013b) and electrochemical workstation (Ge et al., 2012c). Thus, in this work, a novel paper-based MIP-grafted multi-disk micro-disk plate (P-MIP-MMP) was designed and fabricated to demonstrate the high-throughput, sensitive, and specific CL detection of 2,4-D, a proof-of-concept analyte, on Lab-on-paper devices. Like the zones in conventional paper microzone plate (Carrilho et al., 2009) and MIP-based μ-PAD (Ge et al., 2013b), each MIP-grafted paper disk on the P-MIP-MMP can be used to run an independent assay, and the design of the plate facilitates parallel processing of large numbers of samples.

An indirect competitive assay format based on the competition between free 2,4-D in sample solution and immobilized 2,4-D-tobacco peroxidase (TOP) conjugate in grafted MIP layer in the paper disk was employed in this work to quantify the 2,4-D in the sample. TOP (Gazaryan et al., 1998) was chosen as the label due to that this enzyme does not need a CL enhancer for CL detection (Dzgoev et al., 1999). In fact, some of the compounds that are commonly used as CL enhancers are substituted phenols (Ge et al., 2013a; Tu et al., 2012) that might be able to bind to some extent to the grafted MIP layer due to their structural similarity with the analyte 2,4-D. Even though they are added only during the final step of the assay, this might affect assay reproducibility, since this CL detection is based on the MIP bound 2,4-D-enzyme conjugate. Luminol is a widely used CL substrate in combination with peroxidase (Zhang et al., 1999). Unlike the peroxyoxalate systems, luminol requires neither an organic/aqueous mixed solvent system nor a fluorophore for excitation. Therefore, in this work, luminol-TOP-H_2O_2 CL system (Dzgoev et al., 1999) with superior CL property was employed as a model CL system. This novel P-MIP-MMP would open a new door to fabricate low-cost, simple, portable, sensitive, and high-throughput μ-PADs as well as to broaden the potential applications of μ-PADs in environmental and food safety research.

2. Experimental section

2.1. Materials and reagents

Tobacco peroxidase (TOP), 2,4-dichlorophenoxyacetic acid (2,4-D), (3-methacryloxypropyl)-trimethoxysilane, acrylamide (functional monomer), ethylene glycol dimethacrylate (EGDMA, cross-linker), 2,2′-azobisisobutyronitrile (polymerization initiator), 2,4-dichlorophenol (2,4-DCP), 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-chlorophenoxyacetic acid (CPA), and 4-chloro-o-tolyloxyacetic acid (MCPA) were obtained from Sigma (St. Louis, MO). Luminol-H_2O_2 solution used as CL substrate was supplied by Autobio Diagnostics Co. Ltd. TOP-labeled 2,4-D (2,4-D-TOP) was obtained from Zhengzhou Biocell Biotechnology Co., Ltd. (China). Acrylamide and EGDMA were purified before use by an inhibitor remover column (Sigma-Aldrich). All other reagents were of analytical grade and directly used for the following experiments as supplied. Ultrapure water obtained from a Millipore water purification system (resistivity≥18.2 MΩ cm) was used in all assays and solutions. Whatman chromatography paper #1 was purchased from GE Healthcare Worldwide and used with further adjustment of size (square paper disk, 5 mm × 5 mm).

2.2. Fabrication of this P-MIP-MMP

As shown in Scheme 1, prior to the in situ polymerization of MIPs, bare paper disks (Scheme 1A) were first activated using a silane coupling technique to introduce C=C groups on surfaces of cellulose fibers (Scheme 1B) based on the abundant OH groups on per anhydroglucose unit of cellulose (Pelton, 2009). Typically, 100 mg (3-methacryloxypropyl)-trimethoxysilane was dissolved in a 10 mL mixture of ethanol and water (80% ethanol, v/v), and thus was hydrolyzed to form reactive silanol groups. Then paper disks were immersed into the resulting solution and incubated for 1 h at room temperature. Excess silane was then removed by rinsing three times with ethanol, whereafter these paper disks were dried in an air stream.

Secondly, for the in situ polymerization of MIPs in the activated paper disks, a polymerization solution was prepared containing 20 mM EGDMA (cross-linker), 20 mM acrylamide (functional monomer), 0.8 mM 2,2′-azobisisobutyronitrile (polymerization