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Visual electrochemiluminescence biosensing of aflatoxin M1 based on luminol-functionalized, silver nanoparticle-decorated graphene oxide

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ABSTRACT

A sensitive electrochemiluminescence (ECL) aptasensor for aflatoxin M1 (AFM1) detection by a closed bipolar electrode (BPE) array has been introduced. The thiolated AFM1 aptamer was immobilized on gold nanoparticle-coated magnetic Fe_3O_4 nanoparticles (Apt-GMNPs). Luminol-functionalized silver nanoparticle-decorated graphene oxide (GO-L-AgNPs) participates in π-π interactions with the unpaired bases of the immobilized aptamer (Apt-GMNPs-GO-L-AgNPs). After the Apt-GMNPs-GO-L-AgNPs were introduced to a gold anodic BPE array, the individual electrodes were subjected to different concentrations of AFM1. Upon the interaction of AFM1 with the aptamers, the GO-L-AgNPs detach from the aptamer; the resulting ECL of luminol and H_2O_2 at the anodic poles is monitored using a photomultiplier tube (PMT) or smartphone, and the images are analyzed using ImageJ software. This process triggers thionine reduction at the cathodic poles. Under the optimal conditions obtained by a face-centered central composite design (FCCD), the PMT-based detection of the BPE-ECL aptasensor exhibit a linear response over a wide dynamic range from 5 to 150 ng mL^{-1}, with a detection limit of 0.01 ng mL^{-1}. Additionally, smartphone-based detection shows a linear relationship between the ECL image gray value and the logarithmic concentration of the AFM1 target over a range of 10–200 ng mL^{-1}, with a detection limit of 0.05 ng mL^{-1}. Furthermore, the BPE-ECL aptasensor was successfully used to detect AFM1 in milk complex media without any serious interferences with reliable reproducibility (average relative standard deviation (RSD = 2.3%)). This smartphone-based detection opens a new horizon for bioanalysis that does not require a trained technician to operate and is a promising technology for point-of-care testing.

1. Introduction

Aflatoxins (AFs), highly toxic secondary metabolites produced by several fungal species, are the most predominant and toxic mycotoxins and lead to serious human health disorders such as nervous system problems, chronic hepatitis, and liver tumors (Chavarría et al., 2017; Karczmarczyk et al., 2016). Among the identified AFs, aflatoxin M1 (AFM1), with the highest toxicity, has been deemed a primary carcinogen by the International Agency for Research on Cancer (Catanante et al., 2016; Reverté et al., 2016). Considering its extreme toxicity to humans, the maximum limit of AFM1 in milk has been set by the United States and Union European at 0.5 and 0.05 ng mL^{-1}, respectively (Iqbal et al., 2015; Wu et al., 2016). Various methods, including high-performance liquid chromatography-mass spectrometry (Chen et al., 2005; Huang et al., 2014; Rubert et al., 2012) and enzyme-linked immunosorbent assay (ELISA) (Radoi et al., 2008) techniques, have been developed to monitor AFM1. However, the chromatography-based methods suffer from several drawbacks, such as requiring sophisticated instrumentation, trained personnel, and sample preparation methods that are very costly and time-consuming (Eivazzadeh-Keihan et al., 2017). Alternatively, ELISAs, antibody-based immunoassay systems, require high-quality antibodies that are often overly expensive and susceptible to degradation and denaturation in field applications (Malhotra et al., 2014). Specific and highly sensitive immuno- and aptasensors have also been used for AFM1 detection (Bacher et al., 2012; Guo et al., 2016; Istamboulié et al., 2016; Karczmarczyk et al., 2017; Liu et al., 2017; Micheli et al., 2005; Nguyen et al., 2013; Parker et al., 2009; Sharma et al., 2016; Vig et al., 2009), but these measurements are made using external electronic readers, which require a signal converter and data processing unit. Therefore, their use, especially in resource-limited environments and emergency situations, is not practical (Du et al., 2016; Kong et al., 2016; Liu et al., 2016a). In light of the demand for simple, rapid, and cost-effective diagnostics with high sensitivity and selectivity, an electrochemiluminescence (ECL) approach based on bipolar electrochemistry (BE) could help to circumvent these disadvantages (Bouffier et al., 2016; Chow et al., 2009;

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Khoshfetrat et al., 2015; Phuakkong et al., 2016). The basic operating principles of BPEs, along with many interesting applications, have been recently reviewed (Crooks, 2016; Fosdick et al., 2013; Koefoed et al., 2017; Sequeira et al., 2016). Briefly, when a sufficient voltage is applied through an electrolyte solution containing conducting an object (bipolar electrode (BPE)), faradaic reduction at the cathodic pole and oxidation at the anodic pole occur simultaneously (Dorri et al., 2014; Moo et al., 2017). The current flowing through the BPE can be monitored visually via the generated ECL (Bouffier et al., 2016; Khoshfetrat et al., 2015; Liu et al., 2016b; Oja and Zhang, 2016; Zhang et al., 2016) or the electrodissolution of an Ag film (Chow et al., 2010; Fosdick and Crooks, 2011; Termebaf et al., 2015). The intriguing properties of BPE-ECL, including the i) wireless feature of the BPE, ii) lack of a required external light source for ECL, iii) lack of scattered light in the samples compared with fluorescence techniques, iv) ability to control the time and position of the emitted light, and v) lack of excitation source fluctuations, make this strategy suited for miniaturized high-throughput detection through visualization of the ECL signals by a BPE array (Wu et al., 2012).

Various signal-amplification-based ECL methods such as nanoparticle (Chai et al., 2010; Fan et al., 2017b; Zhou et al., 2016), enzyme (Chen et al., 2011) and enzyme-free (Li et al., 2015) techniques, have been developed. In enzyme-based approaches, such as rolling circle amplification (RCA) (Zhang et al., 2017; Zhao et al., 2008) and loop-mediated isothermal amplification (Yuan et al., 2014), the conformation of the conjugated enzymes is usually altered, which, in turn, affects the bioactivity of the enzymes, and the enzymes can be denatured under certain experimental conditions such as high pH and temperature (Fan et al., 2017a). The enzyme-free, assisted methods, i.e., hybridization chain reaction (HCR), (Chen et al., 2012), are complex, time-consuming, and expensive and have low efficiency and sensitivity (Ding et al., 2015). Nanoparticle-based ECL offers a promising alternative in which the nanoparticles are used as labels for the detection of bio/molecules (Rampazzo et al., 2012). Recently, the luminol-functionalized nanoparticles, which exhibit ECL, have attracted much interest owing to their excellent catalytic properties and high loading capacity for reagent molecules with high sensitivity (Xiao et al., 2017). These particles have many useful other properties, including low sample and reagent consumption (Jiang et al., 2015; Khoshfetrat et al., 2015; Zhang et al., 2013). Earlier studies have demonstrated that the ECL intensity of luminol can be enhanced by graphene because of the larger surface area onto which more luminol-functionalized nanoparticles can be loaded (Zhao et al., 2015).

Herein, a sensitive smartphone-based closed BPE-ECL visual array was applied for the detection of AFM1 using luminol-functionalized, silver nanoparticle-decorated, graphene oxide (GO-L-AgNPs) as a signaling probe and gold-coated, magnetic nanoparticles (GMNPs) as both the separation/enrichment tool and the immobilization support for the aptamer (see schematic illustration). Briefly, the aptamer-conjugated GMNPs (Apt-GMNPs) were mixed with the GO-L-AgNPs, resulting in π-π interactions between the unhybridized parts of the aptamer and GO (Apt-GMNPs-GO-L-AgNPs). Then, the anodic poles of the BPE array were exposed to Apt-GMNP-GO-L-AgNPs under a magnetic field below the BPE array. After the introduction of AFM1 at each individual anodic pole, the GO-L-AgNPs nanocomposite releases from Apt-GMNPs surface as a result of the strong interaction between the aptamer and AFM1. Under a sufficient electric field across the electrolyte solution, the ECL reaction occurs on the accumulated GO-L-AgNPs at the anodic poles, and the emission of luminol in the presence of H_2O_2 is followed using a photomultiplier tube (PMT) or smartphone. Concurrently, thionine is reduced at the cathodic poles. Under the optimal conditions, the BPE-ECL system exhibits a linear response over a wide dynamic range of the AFM1 concentration from 10 to 200 ng mL^{-1}, with a detection limit of 0.05 ng mL^{-1}. In this study, multivariate optimization was used to simultaneously assess the effects of the variables and their interactions on the detection strategy (Scheme 1).

2. Experimental

The details of materials, reagents, and instrumentation for physiochemical characterization are given in supporting information (SI). Synthetic AFAS3 aptamer was purchased from Eurofins/MWG/Operon (Germany) with the following sequence: 5′-SH–(CH2)6-ATC CGT CAC ACC TGC TCT GAC GCT GGG GTC GAC CCG GAG AAA TGC ATT CCC CTG TGG TGT TGG CTC CCG TAT-3′. Stock solutions of the oligonucleotides were prepared using 1x phosphate buffer solution (PBS 1x, pH = 7.4) containing 0.01 mol L^{-1} Na_2HPO_4, 0.002 mol L^{-1} KH_2PO_4, 0.15 mol L^{-1} NaCl and 0.15 mol L^{-1} KCl and were stored frozen at −20 °C.

2.1. Synthesis of the Luminol-functionalized GO-L-AgNP nanocomposite

The GO-L-AgNP nanocomposite was synthesized based on the procedure described in the literature (Zhao et al., 2015). First, 2 mL of 5 mmol L^{-1} $AgNO_3$ and 0.33 mL of 1 mg mL^{-1} aqueous GO solution were added to an ethanol/H_2O solution (9:5 V/V). Then, 0.5 mL of 0.01 mol L^{-1} luminol was quickly added to the mixed solution under stirring and allowed to react for 4 h at room temperature. The resulting deep yellow solution was stored at 4 °C.

2.2. Synthesis of gold-coated Fe_3O_4 NPs (GMNPs)

The GMNPs were synthesized in accordance to our previous study (Khoshfetrat and Mehrgardi, 2017). The details of the preparation and synthesis of GMNPs can be founded in Supporting information.

2.3. Preparation of aptamer-conjugated GMNPs

The Apt-GMNPs were prepared via sulfur-gold chemistry. The S-S bond of the aptamer was deprotected by the addition of 20 µL of 0.5 mmol L^{-1} TCEP to a 100 µL aliquot of 6 µM aptamer in the dark for 1 h. Next, 12.5 mg of the GMNPs was added to 100 µL of the pretreated aptamer aliquot and kept for 24 h to form the aptamer self-assembled monolayer on the GMNPs surface. The Apt-GMNPs nanoconjugates were magnetically separated, washed with 1x PBS (pH = 7.4) to remove the unbound aptamers, and resuspended in 1.0 mL of 1x PBS (pH = 7.4) before further modification.

2.4. Interaction of the GO-L-AgNPs with Apt-GMNPs (Apt-GMNP-GO-L-AgNPs)

The adsorption of GO onto Apt-GMNPs occurs via π-π stacking between the GO nanosheets and the free-bounded aptamer bases. A 1x PBS solution (100 µL, pH = 4.6) containing 0.1 mg mL^{-1} GO-L-AgNPs was added to the precipitated Apt-GMNPs. After 1 h of incubation at 4 °C, the magnetically separated Apt-GMNP-GO-L-AgNPs were washed with 1x PBS to remove the unbound GO-L-AgNPs.

2.5. Preparation of the gold BPE array

The details of the fabrication of the BPE array using small pieces of a gold-CD-R followed previously reported procedures (Khoshfetrat et al., 2015). The procedure details for the fabrication of BPE array and fabrication of cell has been described in the Supporting information.

2.6. Modification of the anodic poles

First, 20 µL of Apt-GMNP-GO-L-AgNPs was pipetted onto the anodic poles of the individual gold BPE array under a magnetic field below the surface electrode. The ECL signal of the luminol/H_2O_2 was monitored as an analytical signal using a Samsung S4 smartphone or PMT in a dark room. The ECL signals were recorded using a PMT detector at the set potential of 800 V. The images were captured by instant X software