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A reusable aptamer-based evanescent wave all-fiber biosensor for highly sensitive detection of Ochratoxin A

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ABSTRACT

Although aptamer-based biosensors have attracted ever-increasing attentions and found potential applications in a wide range of areas, they usually adopted the assay protocol of immobilizing DNA probe (e.g., aptamer, aptamer-complementary oligonucleotides) on a solid sensing surface, making it critical and challengeable to keep the integration of nucleic acid surface during the regeneration and the restoration to its original DNA probe form after repeated uses. In order to address the issue, we report a novel aptamer-based biosensing strategy based on an evanescent wave all-fiber (EWA) platform. In a simple target capturing step using aptamer-functionalized magnetic microbeads, signal probes conjugated with streptavidin are released and further detected by a EWA biosensor via a facial dethiobiotin–streptavidin recognition. Apart from the inherent advantages of aptamer-based evanescent wave biosensors (e.g. target versatility, sensitivity, selectivity and portability), the proposed strategy exhibits a high stability and remarkable reusability over other aptasensors. Under the optimized conditions, the typical calibration curve obtained for Ochratoxin A has a detection limit of 3 nM with a linear response ranging from 6 nM to 500 nM. The dethiobiotin–streptavidin sensing surface instead of the traditional nucleic acid one can be reused for over 300 times without losing sensitivity.

1. Introduction

By virtue of their high sensitively, fast kinetics, high selectivity, and facial synthesis (Haun et al., 2010; Song et al., 2010; Zhang et al. 2011), DNA-based biosensors have attracted much attention and witnessed their wide application from diagnostics to basic research (Iliuk et al., 2011; Lee et al. 2010; Li et al., 2010; Shao et al., 2012; Wang 2006). Wherein, the aptamer-based biosensor technology is a rapidly developing area which is anticipated to be competitive with immunoassays and other analytical counterparts currently in use (Citartan et al., 2012; Han et al., 2010; Zhou et al., 2010). Aptamer is a single-stranded oligonucleotide that has been screened through an in vitro selection process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment) (Ellington and Szostak, 1990). As a biological recognition element, aptamer is a promising substitution for antibody because of its clear advantages such as simple production, easy storage, good reproducibility and particularly target versatility (e.g., ranging from small organic molecules to heavy metals, proteins, cells, and even intact viral particles) (Minunni et al., 2004; Tombelli et al., 2007; Zhang et al. 2011). Up to now, numerous optical (Spiridonova and Kopylov, 2002; Zhu et al., 2006), electrochemical (Baker et al., 2006; Wen et al., 2011; Wu et al., 2010), and other aptamer-based (Basnar et al., 2006; Li et al., 2007; Liss et al., 2002) sensors have been developed.

Among them, evanescent field fluorescent biosensors have received widespread attention due to their easy access to miniaturization, highly-sensitive and selective sensing (Leung et al., 2007; Wang and Wolfbeis, 2013), wherein the optical fiber is adopted as one of the most promising transducers. In this transducer, light is propagated down the optically denser medium of fiber core by the total internal reflection (TIR), thus generating an electromagnetic wave (i.e., evanescent field) at the interface of the fiber core and the adjacent less dense medium (e.g. aqueous solution samples). The amplitude of the evanescent wave decreases exponentially with distance into the lower refractive index material, which provides the selectivity to only excite the fluorophores adsorbed, adhered, or bound to the fiber surface. The exponential decay of field strength essentially confines transducible optical signals to within a discrete distance from the waveguide's surface (usually with effective depth of 100–200 nm) (Long et al., 2014), minimizing interference or contribution from components in the bulk phase of lower index medium, thus more sensitive to the targets (Taitt et al., 2005; Zhou et al. 2014). Furthermore, the

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excited light is totally reflected away from the detection region, therefore making easily discriminating the fluorescence signal from the excited light and achieving high sensitivities and low detection limits (Farré et al., 2009; Golden et al., 1992; Taitt et al., 2005; Wadkins et al., 1998). Due to the many advantages, researches on the aptamer-based evanescent wave fluorescence biosensors are blooming in very recently (Long et al., 2014; Yildirim et al., 2012).

Like other biosensors, aptasensors are usually in the form of electrodes, chips, and crystals; hence, immobilization of DNA probe (e.g., aptamer, aptamer-complementary oligonucleotides) on the solid phase is essential in the design of aptamer sensors (Bier et al., 1997; Chuang and Shih, 2001; Liu and Tan, 1999; Piunno et al., 1995; Wu et al., 2007; Zhai et al., 1997), which ensures higher sensing sensitivity and easier device integration compared with homogeneous strategies (Balamurugan et al., 2008; Cosnier and Mailley 2008). Theoretically, device reusability is one of the natural benefits that come with the immobilization strategy, which enables the device easy to achieve portability, automation, and simplicity of use. Moreover, reusability of biosensor is a critical premise to realizing accurate quantification if the chip is not as cheap as a disposable one. However, some biological activity or appropriate orientation of those aptamers might be lost during/after the localization and other regenerating steps, making direct immobilizing protocols disfavored for reusable detections. Many strategies have been reported to meet this challenge, including the use of concentrated salt solutions (Radi et al., 2005), acid/basic solution (Bier et al., 1997; Dave et al., 2010; He et al., 2013; Minunni et al., 2004; Schlensog et al., 2004; Wu et al., 2007), temperature (Freeman et al. 2009; Piunno et al., 1995), chelating agents such as EDTA (Liss et al., 2002), surfactants such as 90% formamide in a TE buffer (10 mM Tris–HCl, pH 8.3, 1 mM EDTA) (Liu and Tan, 1999), sodium dodecyl sulfate (Lai et al., 2007), and thiol-disulfide exchange chemistry (Moore et al., 2007). In all cases above, the typical procedure for biosensor regeneration is to wash aptamers first until the bound target species are removed, then treatment with the regeneration solution, followed by a final rinse with buffer (Balamurugan et al., 2008). Although much effort has been devoted into the development of reusable aptamer-based biosensors, there are few platforms robust enough to be reused for more than 15 times. The restoration to the original DNA probe form is admitted difficult after repeated uses (Liu and Tan, 1999); therefore, how to improve the reusability of aptamer-based biosensor becomes one of the biggest challenges pending for solving in future.

Motivated by the above studies, here we report a novel aptamer-based evanescent wave all-fiber (EWA) biosensing strategy. This strategy is based on aptamer-modified magnetic beads (MB-Ap), STV-conjugated aptamer-complementary DNA oligonucleotides as signal probes (Sp) and a dethiobiotin-modified fiber which is embedded inside the evanescent wave biosensor system. In a simple target capturing step, Sp are released and further detected by a EWA biosensor via a facial dethiobiotin–streptavidin recognition. Therefore, apart from the inherent advantages of aptamer-based evanescent wave biosensors (e.g. target versatility, sensitivity, selectivity and portability), the proposed technique also exhibits high reusability over other aptasensors. To testify the detection protocol, Ochratoxin A (OTA), a widespread food contaminant which showed neurotoxicity, liver toxicity, renal toxicity, teratogenicity and immunetoxicity (Hayat et al., 2013; Rhouati et al., 2013), was chosen as the analyte.

2. Experimental

2.1. Chemical and reagents

Carboxyl-coated magnetic beads (BioMag® Plus Carboxyl, Catalog Code: BP618, 1.5 μm in average diameter) were purchased from Bangs Laboratories Inc. (Fishers, IN). Amicon-3K/10K/50K centrifugal filters were purchased from Millipore Inc. (Billerica, MA). Glutaraldehyde, Streptavidin (STV), Carbodiimide (EDAC), Sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC), 3-Aminopropyl-triethoxysilane (APTS), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), Ochratoxin A (OTA), Aflatoxin B1 (AFT B1), Aflatoxin B2 (AFT B2), Deoxynivalenol (DON) and Chloramphenicol (CHL) were purchased from Sigma–Aldrich, Inc. (St. Louis, MO). Other chemicals used for buffers and solvents were purchased from J&K, Inc.

The following oligonucleotides were purchased from Takara Biotechnoly (Dalian, China) Co., Ltd (from left to right: 5′ to 3′):

DNA for immobilization onto magnetic beads (Aptamers, Ap):
NH_2-AAAAAAGATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA
NH_2-AAAAAAGATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA-FAM
DNA for signal probe (Sp) synthesis (Thiol-DNA-Cy5.5):
SH-AAAAAAAAAAAATGTCCGATGCTC-Cy5.5
Buffers used in this work:
Imidazole Buffer: 0.1 M 1-Methylimidazole, pH 7.0
Prehybridization Buffer: 0.1 M Tris, 0.005 M EDTA, 0.5% N-Laurylsarcosine, 1% BSA, pH 7.4
Hybridization Buffer: 10 mM Tris, 1.0 mM EDTA, 0.01% Tween-20 and 1 M NaCl
Buffer A: 0.1 M NaCl, 0.1 M sodium phosphate buffer, 0.05% Tween-20, pH 7.3
Buffer B: 10 mM PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4), 4 mM $MgCl_2$, pH 7.4,
Wash buffer: 0.5% SDS, pH 1.9

All buffers were prepared either in DEPC-treated deionize water or biomolecule grade deionize water (RNA Nuclease, DNA Nuclease free).

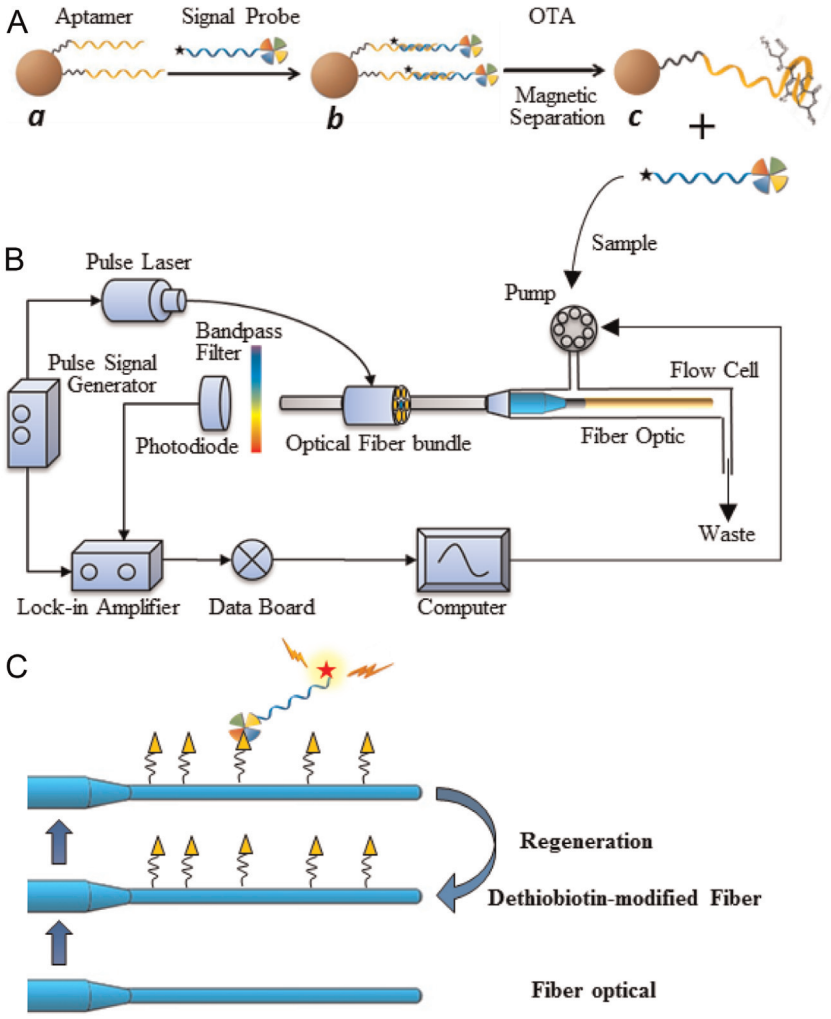
Fluorescence spectrophotometer used in this study: Hitachi F7000 (Hitachi Ltd., Japan)

2.2. Preparation of functional magnetic beads

800 nM aptamer (Ap) and 0.1 M EDC were gently mixed in 2 ml imidazole buffer. The mixture were immediately incubated with 20 mg carboxyl magnetic beads (MBs) at room temperature for 24 h to allow the formation of MB-Ap. At the end of the coupling reaction, MB-Ap were treated with prehybridization buffer at 68 °C for 4 h to block unreacted amino groups on the surface. 2 mg synthesized Sp (see supplementary material for the synthesis and PAGE characterization of the signal probes) were added to washed MB-Ap in 2 ml hybridization buffer, and the mixture were kept on a rotator for 2.5 h at room temperature for the formation of MB-Sp. After hybridization, MB-Sp were washed and preserved in 2 ml PBS buffer (10 mM), diluting the final solids content to 10 mg/ml.

2.3. All-fiber evanescent wave biosensing platform

Scheme 1B presents the schematic of a EWA biosensing platform which has been proposed as our previously described (Long et al. 2008) with a significant modification. Briefly, a 635 nm, 10 mW pulse diode laser with a pigtail was coupled into a multimode fiber probe through an optical fiber bundle. The incident light propagated along the fiber probe via TIR. The evanescent



Scheme 1. (A) Preparation of functional magnetic beads (MBs) and OTA-induced release of signal probes (Sp). a: aptamer–modified MBs (MB-Ap), b: Sp modified magnetic beads (MB-Sp), c: OTA-binded MBs; (B) Schematic of a EWA biosensing platform; (C) The capture of streptavidin (STV)-conjugated Sp onto a dethiobiotin-modified fiber via STV-dethiobiotin recognition for further EWA detection and the sensing surface regeneration via a simple SDS treatment.

wave generated at the probe surface interacted with the surface-bound fluorophores, thus inducing their excitation. After that, the emitted fluorescence was collected by the same optical fiber bundle, and subsequently filtered by means of a band pass filter and detected by photodiodes through lock-in detection. The probe fiber was embedded in a glass flow cell with a flow channel having a nominal dimension of 55 mm in length and 2 mm in diameter. All reagents were delivered by a flow delivery system operated with a peristaltic pump. The controls of fluid delivery system and data acquisition and processing were automatically performed by the built-in computer.

In this system, an optical fiber bundle instead of the previous single-multi fiber optic coupler was used for the transmission of the excitation light and the collection and transmission of fluorescence. The optical fiber bundle was composed of a single mode optical fiber surrounded by six multimode optical fibers (A cross-sectional schematic view of the optical fiber bundle was shown in Fig. S2). Apart from the inherent advantages of reduced optical components and no optical alignment required (Long et al. 2008), this new EWA biosensor exhibited cheaper, more easy-to-get, more compact and consistent, enabling sensitive detection for environmental samples.

2.4. Modification of optical fiber

The plastic-clad step-index silica optical fiber with the length of 8.5 cm and core diameter of 600 μm ($NA=0.22$) was used in the study. The fiber cladding with length of 5.0 cm in the distal end was stripped away to expose the fiber core. In order to reduce the loss of fluorescence signal level coupling back into the fiber, the straight fiber core was tapered using the tube-etching method (Long et al., 2008) to form a combination tapered fiber type (Scheme 1C), which was confirmed more sensitive than the continuous tapered fiber type as stated in the previous reports (Golden et al., 1992; Nath and Anand, 1998). As the sensitivity of evanescent wave fiber-optic sensor heavily depends on the core diameter in the sensing region (Golden et al. 1992; Jiao et al. 2013; Nath and Anand, 1998), the tapered ratio of the fiber probe was theoretically and experimentally optimized to be approximately 0.37 in the study. In order to establish a reusable fiber surface without depriving the binding properties of the immobilized molecules, dethiobiotin molecules were immobilized on the tapered region to form the biosensitive layer, which are shown in the following steps (Fig. 1).

Initially, the fiber was immersed in piranha solution ($H_2SO_4/H_2O_2=3:1$, v/v) at 100 °C for 1 h to remove possible

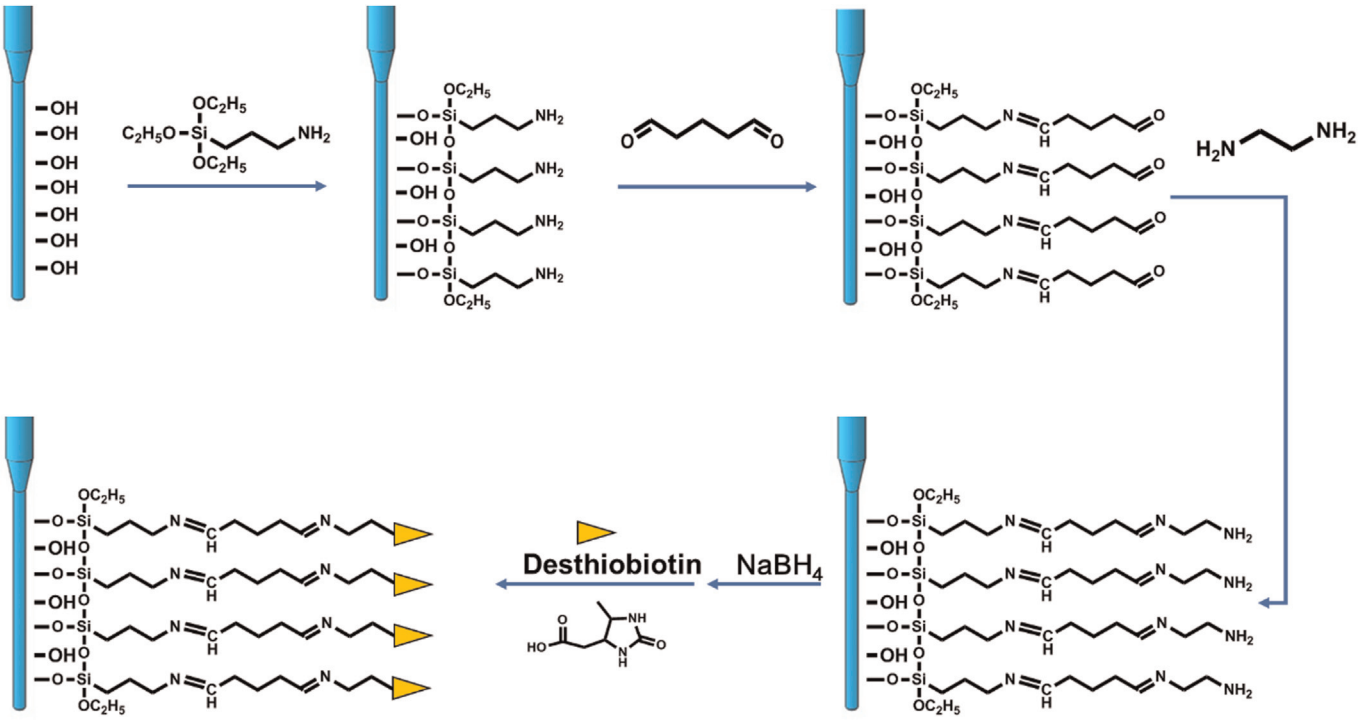


Fig. 1. Schematic diagram for the surface modification of optical fiber.

contaminants and introduce hydroxyl group onto the sensing surface of the fiber. Then the hydroxyl-modified fiber was washed with deionized water and dried in N_2. Next, the fiber was rinsed thoroughly into 2% (v/v) APTS/toluene solution for 1 h before being washed with dry toluene and dried in N_2. The amino groups on the fiber were allowed to react with 2.5% (v/v) glutaraldehyde in ethanol for 1 h at room temperature. After being washed 10 times with ethanol, the fiber was immersed into 10% (v/v) ethylenediamine/ethanol for 1.5 h at room temperature. The unreacted aldehyde groups were reduced using 1 mg/ml $NaBH_4$ in ethanol for 15 min.

Meanwhile, 25 mg Dethiobiotin, 50 mg EDC and 50 mg NHS were dissolved in 1 ml DMF, and the obtained mixture was rotated at room temperature for 3 h. At the end of the rotation, 200 µl $NaHCO_3$–Na_2CO_3 buffer (1 M, pH 8.7) was added to the mixture. Subsequently, the amino modified fiber was rinsed into the abovementioned mixture for incubation at room temperature overnight. Finally, the non-specific binding sites on the fiber surface were blocked by immersing the fiber into the BSA solution (2 mg/ml) for 1 h. The prepared fiber was stored at 4 °C until use.

2.5. OTA detection and biosensor regeneration

In each test, if not specified, 600 µL buffer B containing 0.4 mg MB-Sp were incubated with different concentrations of OTA for 15 min at room temperature to induce the Sp release. After magnetic separation, the supernatant solution was collected as the test sample of EWA. The laser was switched on at the beginning of the EWA measurement. Buffer B was pumped through the flow cell for ~1 min to ensure a stable baseline was achieved before the sample detection. Typically, for an instrumental EWA detection, the peristaltic pump was run at 1.5 ml/min to ensure sample delivery to the flow cell. Once the cell was filled with the test sample (usually 30 s), the pump was off for 1.5 min for the recognition reaction on dethiobiotin-modified optical fiber to take place. Meanwhile, the EWA signals were recorded and related to the corresponding OTA. Between each detection, the captured Sp were eluted from fiber-optic surface with ~1.5 ml wash buffer in 1 min to ensure the regeneration of sensing surface. The whole EWA detection procedure takes less than 5 min.

2.6. Recovery in oat samples

In order to evaluate the practical applicability of this technique, the EWA biosensor was used for determination of OTA in oat samples. Non-contaminated commercial raw oats were purchased from the local supermarket. Firstly, 0.5 g oat sample was mixed with 10 mL of extraction solvent (methanol:water=7:3, v/v) containing various concentrations of OTA. The mixture were gently mixed on a rotator for 30 min at room temperature. Subsequently, after centrifuging at 12,000 rpm for 10 min, the extract was passed through a 0.45 µm syringe filter and diluted with buffer B to final OTA concentrations at three levels (7.5 nM, 10 nM, and 15 nM) for the recovery experiments.

3. Results and discussion

3.1. Experimental principle of the aptamer-based EWA biosensor

Aptamers exhibit high target-binding affinity because their binding function is largely dependent upon stable secondary structure interactions (Kirby et al., 2004; Zhou et al., 2010); however, it is challengeable to keep the restoration to the original DNA probe form after repeated uses (Liu and Tan, 1999). Inspired by the immunosensors undergoing the antibody–antigen recognition which feature with highly reusability of up to 300 times of regenerated test cycles (Rodriguez-Mozaz et al., 2005; Zhou et al., 2014), we describe here a novel aptamer-based EWA biosensing strategy as shown in Scheme 1.

Firstly, the specific OTA aptamers (Ap) (Cruz-Aguado and Penner, 2008a, b) were assembled onto MBs. Sp, which were designed

to hybridize with Ap and response to the latter structure switching, were immobilized onto MBs by Ap/Sp hybridization. The existence of OTA would induce the structure switching from an Ap/Sp duplex into an Ap/target complex. As a result, Sp were forced to dissociate from the MBs and were released into the supernatant solution (Scheme 1A).

Such a conformational change essentially increased the concentration of Sp in the supernatant. To analyze this change, the supernatant was injected into the flow cell of a EWA biosensor after magnetic isolation (Scheme 1B). During the followed EWA detection (Scheme 1C), a dethiobiotin-modifed fiber would capture Sp via the classic dethiobiotin-STV recognition. Once captured within the evanescent wave-field, the Cy5.5 fluorophores labeled on Sp would emit its unique fluorescence, leading to a detectable EWA signal. After that, a simple SDS eluting was applied as a sensor regeneration step, allowing the reuse of sensing surface without losing in sensitivity. However, in the absence of OTA, Sp held onto MBs and were not available in the supernatant, resulting in extremely low EWA signal. By setting up the relationship between net EWA signals and OTA concentrations, a reusable aptamer-based EWA biosensor for highly sensitive detection of OTA was developed.

In the proposed strategy, the more complex tertiary structural interactions between STV and dethiobiotin was adopted instead of DNA hybridization or aptamer-target affinity in the solid phase in the traditional aptasensors (Bier et al. 1997; Chuang and Shih 2001; Liu and Tan 1999; Piunno et al. 1995; Wu et al. 2007; Zhai et al. 1997). The affinity constant (Kd) of STV-dethiobiotin is 0.5 pM (Garlick and Giese 1988), comparable to those of some antibodies and their antigens (Lee et al. 2004; Rispens et al. 2013; Schier et al. 1996; Smith et al. 2008) and suitable for the regenerated affinity biosensing.

3.2. Identification of functional magnetic beads

To confirm the formation of MB-Ap, the supernatant fluorescence data before and after coupling reaction was compared by introducing 3′-FAM-Ap. Fluorescence spectroscopies (Fig. 2A) shows a decline in FAM emissions intensity, indicating that little Ap were left in the supernatant solution and it was difficult to wash these oligonucleotides off from MBs. As observed from the fluorescence microscopy images (Fig. 2B), pre-coupling MBs were non-fluorescent (Fig. 2B, i–ii), and at the beginning, FAM fluorophores largely appeared in solution (Fig. 2B,iii–iv). After coupling reaction and a regular washing step, MBs in an experiment group remained fluorescent (Fig. 2B,vii–x), while MBs in a control group lost nearly all fluorescence (Fig. 2B,v–vi). Moreover, the coupling chemistries here were also proved to have extremely low levels of nonspecific binding. These results together proved the formation of MB-Ap.

To confirm the formation of MB-Sp, the supernatant fluorescence data before and after hybridization reaction was compared. Fluorescence spectroscopies (Fig. 2C) showed a decline in Cy5.5 emissions intensity, indicating that most Sp were hybridized to MB-Ap and little were left in the supernatant solution.

3.3. Optimization of experimental conditions

Experiment variables affecting the biosensor performance were optimized. As shown in Fig. 3, first, the Mg^{2+} concentration in buffer B was investigated as it might influence the OTA binding

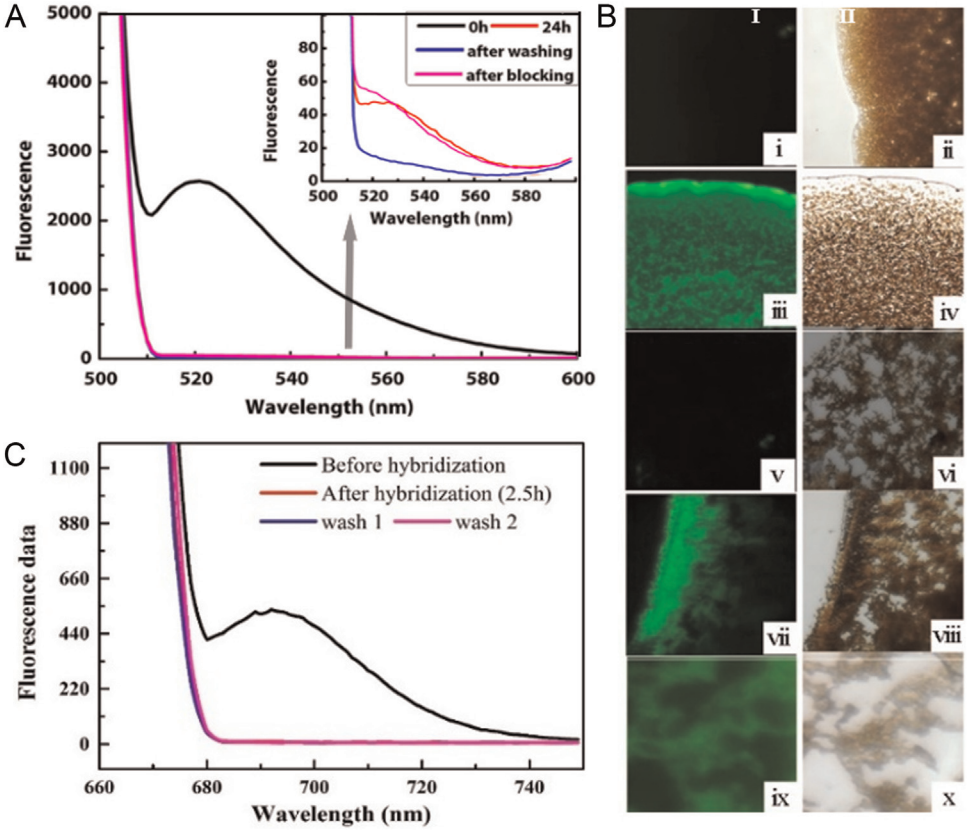


Fig. 2. Characterization of functional magnetic beads. (A) Fluorescence spectroscopy before and after attaching 3′-FAM-Ap to MBs. Inset: partial enlarged view of the low fluorescence district. (B) Attaching 3′-FAM-Ap to MBs. Fluorescence microscopy images (Column I) and corresponding bright field microscopy images (Column II). i–ii: Pre-coupling MBs. iii–iv: Post-coupling MBs (without washing). v–vi: Post-coupling MBs in a control group (after washing), in which EDC was omitted from the reaction mixture. vii–x: Post-coupling MBs in an experiment group (after washing). (C) Fluorescence spectroscopy before and after hybridization.

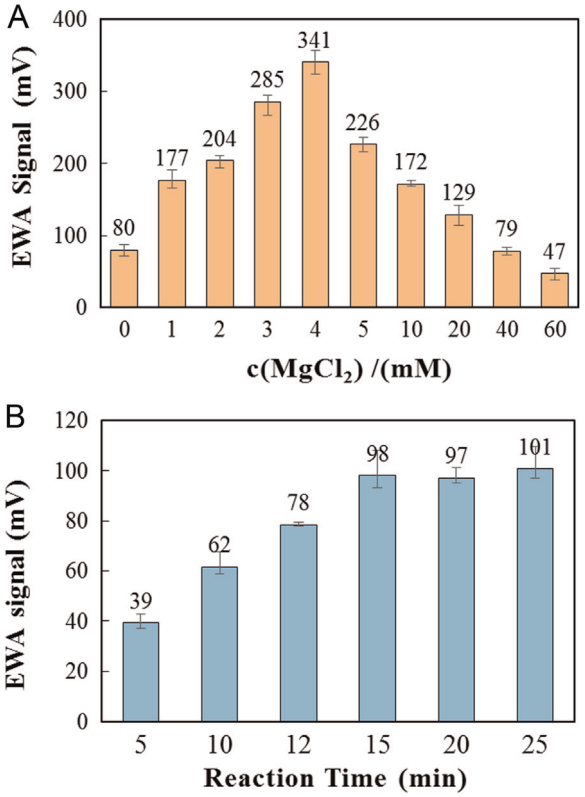


Fig. 3. Optimization of experimental conditions. (A) Net EWA signal response of the system in the presence of OTA (500 nM) when incubated into 10 mM PBS buffer with various concentration of $MgCl_2$. (B) Net EWA signal response of the system in the presence of OTA (10 nM) as a function of incubation time.

procedure (Cruz-Aguado and Penner, 2008b). Fig. 3A shows the EWA signals responding to 500 nM OTA by incubating functional MBs in Buffer B with different Mg^{2+} concentrations. The result shows that the optimum Mg^{2+} concentration for the OTA detection is 4 mM.

The incubation time for OTA binding was also investigated. Fig. 3B shows the EWA signals responding to 10 nM OTA by incubating functional MBs in Buffer B (with 4 mM $MgCl_2$) for different times. The signal increased with increasing the reaction time and reached equilibrium over ~15 min, which was set as the standard reaction time in the following experiments.

3.4. Detection performance of the EWA biosensor

3.4.1. Detection

As shown in Fig. 4A and B, calibration curves were measured by relating the EWA signals to the corresponding OTA concentrations. All values given in the figure plots represent the mean values of three individual measurements and their standard deviations. Curves were fitted with a four-parameter logistic model (Long et.al, 2008), signals from 20% to 80% of the maximal signal were used to define the working range. Typical calibration curve obtained for OTA had a detection limit of 3 nM (1.2 ng/ml). A linear response for OTA ranged from 6–500 nM ($y=151.45x-49.253$, $R^2=0.9982$). According to the regulations introduced by European Union, the maximum permitted levels of OTA in food stuffs are 5 μg/kg for cereals and coffee products, and 2 μg/L for wines and grape-beverages. Therefore, with a proper sample pre-treatment, this method is qualified to detect real environmental samples. Moreover, the analytical performances of different optical aptamer-based assays developed for OTA analysis are summarized and the sensitivity of this proposed method

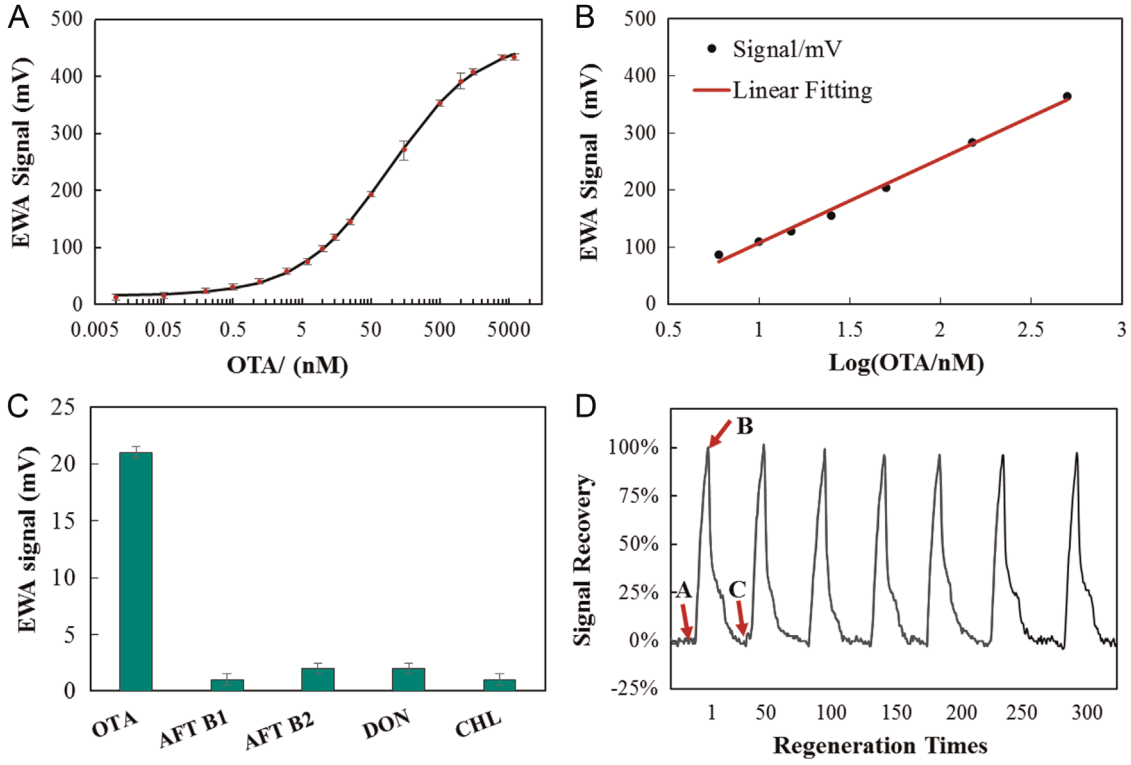


Fig. 4. (A) The calibration curve for determination of OTA concentration using the aptamer-based EWA system. (B) Linear plot of the change in EWA signal vs. OTA concentration. (C) Selectivity of the aptamer-based EWA biosensor toward OTA and its analogs. (D) The regeneration of the sensing surface. Point A: sample injection; Point B: the peak EWA signal; Point C: fiber surface regeneration (Each signal value is the average of three independent experimental results).

Table 1
Summary of the optical aptamer-based assays developed for OTA analysis.

Detection method	Linear range (ng/mL)	LOD (ng/mL)	Reference
Colorimetric	1–10	1	Barthelmebs et al. (2011)
Colorimetric	8–250	8	Yang et al. (2011)
Luminescent	0.02–3	0.007	Wang et al. (2010)
Fluorescent	10–80	9.64	Guo et al. (2011)
Fluorescent	1.9–10	1.9	Wang et al. (2011)
Fluorescent	1–100	0.8	Chen et al. (2012)
EWA	2.4–200	1.2	This study

Table 2
Determination of OTA in Oat samples ($n=3$).

Sample	OTA (nM)		Recovery (%)
	Spiked	Found mean[a] ± SD[b]	
Oat 1	7.5	7.8 ± 0.13	104
Oat 2	10	9.0 ± 0.23	90
Oat 3	15	13.9 ± 0.16	92

[a] Mean value of three individual determinations
[b] Standard deviation

is comparable to these techniques (Table 1). We also believe that it is possible to improve its sensitivity by further optimization.

3.4.2. Selectivity

As the EWA signal is essentially based on the target binding-induced dissociation of Sp, the aptasensor described here is specific for the target sensing. By using AFT B1, AFT B2, DON and CHL as OTA analogs, we investigated the selectivity of the aptasensor to OTA over its analogs. Fig. 4**C** compares the net EWA signals after the functional MBs being incubated with those analogs in buffer B. The incubation of the MBs with 0.01 nM analogs did not produce obvious EWA signal, while the incubation of the MBs with the same concentration of OTA produced a clear increase in the EWA signal. This comparison clearly indicated that the proposed aptasensor was very selective for OTA detection.

3.4.3. Regeneration

Owing to the reversible interactions between STV and dethiobiotin molecules, Sp could be washed off easily from the dethiobiotin-modified fiber using 0.5% SDS (pH 1.9) solution. After that, simple incubation into buffer B could regenerate the sensing surface. As shown in Fig. 4D (all being treated with 5 nM OTA), during the whole test procedure, the fiber was reused for at least 300 times and still maintained good performance. The signal recoveries of all measured samples ranged from 96% to 101%. These data confirm that the proposed sensing method is applicable for OTA detection with enough accuracy and reusability.

3.5. Determination of OTA in oat samples

In the recovery experiment, the detection performance of this technique for the spiked oat samples with OTA is presented in Table 2. The average recoveries were found to vary from 90% to 104%, with the satisfactory variations that the relative standard deviation was no more than 3%. Results confirm the satisfactory accuracy of the developed aptamer-based EWA biosensor technique for OTA detection in reality.

4. Conclusions

Featuring the inherent advantages of a broad range of analytes, sensitivity, selectivity and portability, a novel aptamer-based EWA biosensor has been developed with high reusability. The sensing surface via a facial dethiobiotin–streptavidin recognition can be easily regenerated and used for at least 300 measurements with acceptable sensitivity, to the best of our knowledge, which is unprecedented for the aptamer-based biosensors. The regeneration of the biosensing surface is highly facial and repeatable, which not only further strengthen the quantification accuracy of this method, but also enable the biosensor easy to achieve portability, automation, and simplicity of use. Benefitting from the target versatility of the recognition element of aptamer, the proposed EWA biosensor shows great potentials to detect and quantify a broad range of analytes involved food, environment, diagnostics etc. Not only could this biosensor provide a regenerable, novel, cheap, rapid, and robust technique as an alternative to currently available detection approaches, it also shows promising application in multi-target detection and on-site analysis. Besides, this sensing strategy is expected universal and expandable for other solid-phase affinity biosensing technologies if both reusability and target versatility are to be considered. Moreover, this sensing design can be easily integrated with other platforms like microelectronics and microfluidics systems to gain advantages in miniaturization and multiplex detection.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.10.079.

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