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Development of novel portable and reusable fiber optical chemiluminescent biosensor and its application for sensitive detection of microcystin-LR

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ABSTRACT

A novel portable fiber optical chemiluminescent biosensor (FOCB) system was successfully constructed using fiber optic bio-probe as biorecognition element as well as transducer and a Si-based photodiode detector (PD-3000). The compact design of FOCB with free magnetic beads and photomultiplier allows it to be portable and suitable for on-site and automatic detection of targets, as well as to be cost-effective and small sample volume (only 40.0 μL). The sensitivity of 5 fW could be obtained for the FOCB due to the high performance of PD-3000 and no background light signal. Next, the FOCB was applied for the CL-based detection of microcystin-LR (MC-LR) in water using hapten-carrier protein conjugates modified fiber optic bio-probe as biorecognition element. Based on indirect competitive sandwich-like CL immunoassay principle, the highly sensitive detection of MC-LR was achieved using FOCB system. Under optimal conditions, the limit of detection for MC-LR is 0.03 μg/L, which is comparable with that of most reported MC-LR immunoassay methods. The robustness of the hapten–carrier protein-modified biosensor surface allowed multiple MC-LR immunoassays without any significant loss of performance, which ensured accurate and cost-effective detection results. The proposed strategy demonstrated good recovery, precision, and accuracy through the evaluation of the spiked water samples. The FOCB system can be readily extended toward the on-site real-time sensitive detection of other targets in the field of environment, food, and medical diagnosis.

1. Introduction

Cyanobacterial blooms, one of the most pressing global environmental problems, represent a great threat to public health and the water environment due to the release of cyanotoxins. Microcystins (MCs), the most widespread cyanotoxins with a group of bioactive cyclic heptapeptide, are serious hepatotoxins which can strongly suppress the activity of protein phosphatases and result in liver damage and necrosis (Campos and Vasconcelos, 2010). Many cases of animal-poisoning and human health diseases, some of which caused liver cancer and even death, have been reported (Zhang et al., 2011, 2017). Several studies demonstrated that long periods of exposure to microcystins, even at very low concentration, promoted the risk of tumor formation (Amé et al., 2010; Campos and Vasconcelos, 2010; Qileng et al., 2017). Nowadays, more than 90 known microcystin congeners have been found, and most of them have a lethal dose of about 50–600 μg/kg (Welker et al., 2004). Among them, microcystin-LR (MC-LR), containing leucine (L) in position 2 and arginine (R) in positions 4, is one of the most common microcystin congeners. To minimize public exposure to MCs, the World Health Organization (WHO) has set a guideline value of 1.0 μg/L for MC-LR in drinking water. Several detection methods, including high-performance liquid chromatography (HPLC) (Rivasseau et al., 1998), protein phosphatase inhibition assays (PPIA) (Rivasseau et al., 1999), and enzyme-linked immunosorbent assay (ELISA) (Lindner et al., 2004; Sheng et al., 2006; Campàs and Marty, 2007), have been applied for MC-LR detection. HPLC is more precise than the other methods and can distinguish microcystin congeners, while it requires expensive equipment, complex procedure, long analysis time, and trained personnel (Tsuji et al., 1994). ELISA and PPIA are sensitive and effective detection methods but they cannot be used for routine

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screening and may yield false positives if the enzyme is inhibited by other unrelated environmental pollutants. To ensure human health and environmental safety, it is still essential to develop novel techniques for the real-time on-site detection of MC-LR with high sensitivity and specificity due to its low concentration level and large scale analogues.

Chemiluminescence (CL), the luminescence produced from a chemical reaction, has been proved to be a powerful technique for the detection of trace analytes due to its high sensitivity, wide linear range, and low background noise (Williams et al., 2006). Through integrating molecular biotechnology with different transducers, various CL biosensors have successfully applied for medical diagnosis, environmental monitoring, and food analysis (Amstislavski et al., 2012; Zhang et al., 2013; Timofeeva et al., 2017). Because of the absence of an excitation light source and a spectral resolving system, CL biosensor are very suitable for developing a portable detection instrument for the on-site real-time detection of environmental pollutants. However, the conventional CL instruments used magnetic-beads coated with antibodies to capture targets to form immune complex, which needed to be separated from the original solution using magnetic field to eliminate the matrix effect and reduce the background interference. This resulted in the complex structure of the conventional CL instrument and multiple detection steps (Amstislavski et al., 2012; Iranifam, 2013). Moreover, the immune magnetic-beads were not generally reused, leading to high detection cost and inaccurate results. Because most of the CL based reactions have low quantum efficiencies and produce a weak luminescence, the photomultiplier (PMT) are applied for the CL intensity detection (Timofeeva et al., 2017). However, the use of PMT makes CL instrument difficult to be miniature and portable because of its large volume, high price, high polarizing voltage, and sensitive to electromagnetic field interference (Amstislavski et al., 2012; Iranifam, 2013). A chemiluminescence multichannel immunosensor based on a capillary immunoassay technique was used to detect MC-LR, but it employed PMT to detect CL intensity and was semi-quantitative (Lindner et al., 2009).

Herein, a novel portable and reusable fiber optic CL biosensor (FOCB) has been developed for the sensitive on-site detection of MC-LR in water samples. To avoid the deficiency of immune magnetic-beads, coating-antigen modified fiber optical probe in the FOCB system was used as both biorecognition elements and CL transducer. Our previous studies demonstrated that the coating-antigen modified fiber optic bioprobe had a good regeneration performance and could be reused more than 100 times without any loss of activity (Lou et al., 2017; Hao et al., 2015). Meanwhile, the fiber optical probe as a transducer could effectively improve the collection efficiency of CL signal, resulting in a higher sensitivity of CL biosensor. Instead of using PMT, a high sensitive and low noise photodiode (PD) detector was applied for the detection of CL intensity. Compared to PMT, PD detector has advantages of small volume, cost-effective, broad spectral response, and large dynamic range (Geng et al., 2018). Through integrating microfluidic cell and automatic sampling system, the proposed CL biosensor is very suitable for the real-time on-site detection of MC-LR due to its high sensitivity, specificity, portability, and reusability.

2. Experimental

2.1. Immunoreagents and chemicals

Monoclonal anti-MC-LR antibody and coating-antigen MC-LR-OVA were produced by our research group (Long et al., 2009). Goat anti-mouse-HRP secondary antibody was purchased from Thermo Fisher Scientific (Beijing, China). Bovine serum albumin (BSA), 3-mercaptopropyl-trimethoxysilane (MTS), N-(4-maleimidobutyryloxy) succinimide (GMBS) were purchased from Sigma-Aldrich (Steinheim, Germany), the CL substrate (HRP-H_2O_2-luminol system) was purchased from Yesen Bio-Technology Limited (Shanghai, China). Methanol, acetone, acetonitrile, H_2SO_4, H_2O_2, and all the other reagents, unless specified, were supplied by the Beijing Chemical Agents (Beijing, China). These reagents were all of analytical grade and used without further purification. Distilled deionized water was used throughout the investigation. Buffers used in this experiment were 0.01 mol/L phosphate buffered saline (PBS, including 137 mmol/L NaCl, 2.7 mmol/L KCl, 4.3 mmol/L Na_2HPO_4 and 1.4 mmol/L KH_2PO_4, pH = 7.4) and antibody dilution buffer (1.0 g BSA, 0.02 g NaN_3 and 100 mL 0.01 mol/L PBS). MC-LR stock solutions were prepared with PBS and stored at 4 °C. Standard concentrations of the analyte were prepared from the stock solution by serial dilutions in PBS. And all the antibody solutions were diluted by antibody dilution buffer. 0.5% sodium dodecyl sulfate (SDS, pH = 1.9) was used as the regenerated solution.

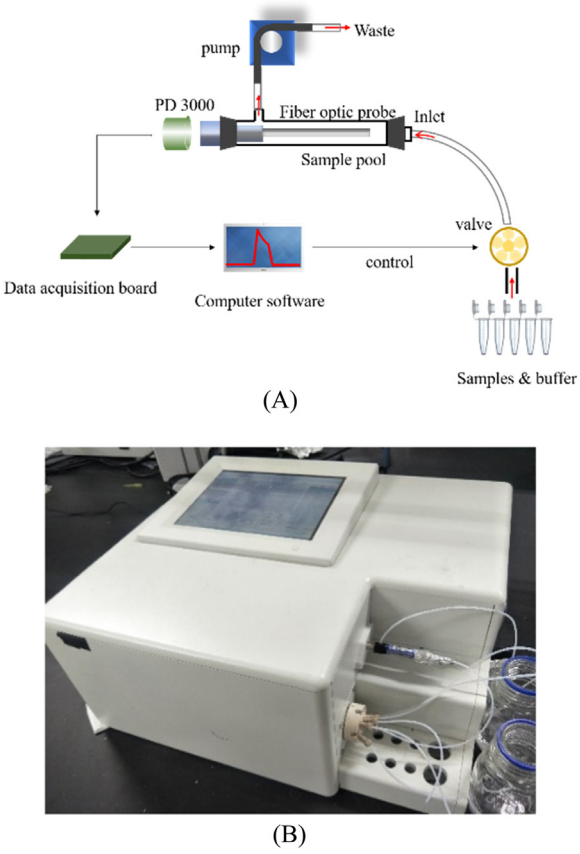

Fig. 1. (A) Schematic of portable fiber optic CL biosensor; (B) picture of the FOCB.

2.2. Design of FOCB

The Scheme of FOCB system for the CL detection is shown in Fig. 1A, and the picture of the FOCB (29.5 × 23.2 × 15.8 cm) were shown in Fig. 1B. In this system, a fiber optical probe (600 μm, NA = 0.22) modified by coating-antigen conjugates (e.g. MC-LR-OVA) was regarded as biorecognition element and embedded in a black cylindrical microfluidic cell (ϕ1.5 mm, 35 mm length, effective volume ~ 60 μL) to block environmental light. Meanwhile, the fiber optic bioprobe was also used as a transducer to collect the CL signal. To simplify the system structure and improve the detection efficiency, a new Si-based photodiode detector PD-3000 developed by our group was directly placed in the end of fiber optical probe to detect the CL signal without any other optical separated elements. The flow sampling system was significant for achieving automated measurement. This system consisted of a peristaltic pump, a six-way injection valve, and a microfluidic cell. Various solutions (e.g. buffer, sample, and regeneration solution) were successively introduced into the microfluidic cell.

All assay relevant parameters were controlled using a special parser embedded within the user interface of the measurement software. Fluid handling and data acquisition were fully automated and computer controlled.

2.3. Preparation of bio-probe for MC-LR detection

Fiber optic bio-probe was prepared using the plastic-clad step-index silica optical fiber (Chunhui Science & technology industrial Co., China) with a numerical aperture (NA) of 0.22, whose core (Φ577 μm in diameter) is surrounded by a silica cladding (refractive index of 1.44 at 633 nm), followed by a buffer layer and a black jacket. The fiber optic bio-probes consist of a 5.5 cm length with the black jacket and buffer layer removed from 3.0 cm along the distal end to form the sensing region. The distal end and cylindrical surface of the fiber optic bio-probe was modified using biorecognition molecules (Fig. S1), which increased the amount of biorecognition molecules on the fiber optic bio-probe. As well known, the CL intensity (I) could be calculated by,

$$I_{CL} = \varnothing_{CL} \cdot dc/dt \quad (1)$$

where I_{CL} is CL intensity, $\varnothing_{CL}$ is the reaction constant, and c is the concentration of reactants. When the other reaction conditions (e.g. H_2O_2, luminol concentration) are the same, the CL intensity is proportional to the concentration of HRP-labeled secondary antibody. Therefore, increasing the amount of biorecognition molecules on the fiber optic bio-probe may increase the concentration of HRP-labeled secondary antibody bound to the fiber optic bio-probe in the CL immunoassay (CLIA) in our detection system. Compared to that only the end surface of the probe is modified by the biorecognition molecules, the amount of biorecognition molecules immobilized on the proposed bio-probe increases 200 times. It should be noted that when the bio-probe was embedded into the cell, the effective volume of cell was about 40.0 μL.

Because MC-LR with a low molecular weight was difficult to immobilize onto the probe surface directly, the hapten-carrier protein conjugates MC-LR-OVA was covalently immobilized on the aminated fiber optic surface to prepare bio-probe according to the method described by our group with a minor adjustment (Long et al., 2014) (Supporting information).

2.4. CL immunosensing mechanism of MC-LR

Fig. 2A and B illustrate the CL immunosensing mechanism of the FOCB system for MC-LR detection and the exemplary signal profile, respectively. The assay format used for all measurements was an indirect competitive sandwich-like immunoassay. One analysis cycle included five steps as followings. First, 50 μL of samples containing various concentrations of MC-LR were mixed and pre-reacted with 50 μL of anti-MC-LR antibodies at a fixed concentration for a certain time. In this process, part of antibodies specifically bound with MC-LR and the occupied antibody binding sites were proportional to the concentration of MC-LR in the samples. Second, the mixture was introduced into the cell after pre-reaction (Step I in Fig. 2A). The antibodies containing free binding sites could bind to the MC-LR-OVA immobilized onto the fiber optic bio-probe surface (Phase I in Fig. 2B). Third, the cell was rinsed with PBS to remove any excess anti-MC-LR antibody, and a certain concentration of the secondary antibodies labeled by HRP was added to the cell (Step II in Fig. 2A) and allowed them to bind with the anti-MC-LR antibody bound to MC-LR-OVA on the sensing surface for several minutes (Phase III in Fig. 2B). Fourth, 100 μL CL substrate solution was delivered into the cell (Step III in Fig. 2A), and the HRP labeled on the secondary antibodies catalyzed the luminescence of substrates (Phase III in Fig. 2B). The CL intensity was on-time recorded. Finally, to reuse the probe, the immunosensing surface was regenerated with 0.5% SDS solution (pH 1.9) for 180 s (Step IV in Fig. 2B) and washed with PBS solution (Phase IV in Fig. 2B). The whole detecting cycle time was less than 30 min.

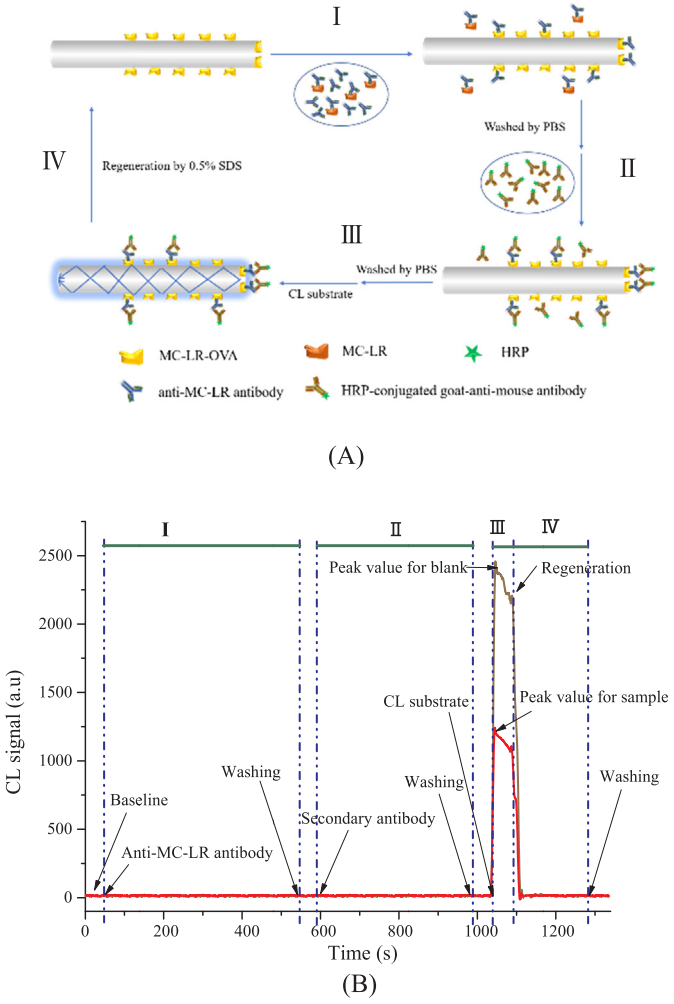

Fig. 2. (A) Schematic of the CL immunosensing mechanism of MC-LR detection; (B) Representative signal profile for MC-LR detection with the FOCB (Pre-reaction and incubation time were 500 s and 300 s, respectively, 1.0 μg/mL anti-MC-LR antibody, 5.0 μg/mL HRP-labeled secondary antibody, the MC-LR concentration for the black curve and red curve is 0 and 10 μg/L, respectively).

Fig. 2B shows the representative real-time signal traces for one analysis cycle. For each assay, the net CL intensity I used for the subsequent determination of dose–response curve was calculated by subtracting the baseline value according to Eq. (2):

$$I = \text{CL intensity at the peak value–the baseline value} \quad (2)$$

The normalized response was calculated according to the following equation:

$$I_n = I_s/I_b (\text{a.u.CLintensity}) \quad (3)$$

where I_b is the net CL intensity of the blank in the absence of MC-LR, and I_s is the net CL intensity of the samples.

3. Results and discussion

3.1. Characterization of FOCB

To evaluate the performance of the proposed FOCB, a MC-LR-OVA modified fiber optic immunosensor used as biorecognition element as well as transducer was embedded into the sample cell to perform MC-LR immunoassay. After 50 μL sample containing an amount of MC-LR and 50 μL 1.0 μg/mL anti-MC-LR antibody solution was mixed for 7 min, and the mixture was introduced into the sample cell for 500 s

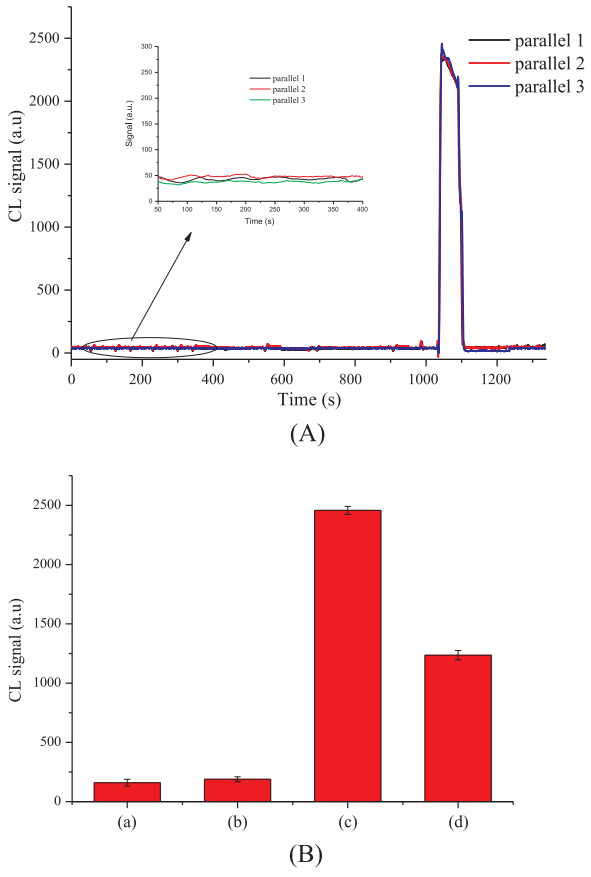

Fig. 3. (A) Characteristics of FOCB system (Pre-reaction and incubation time of the detection were 500 s and 300 s, respectively, 1.0 μg/mL anti-MC-LR antibody, 5.0 μg/mL HRP-labeled secondary antibody). (B) Assessment of the immobilization effectiveness and specific binding between MC-LR immobilized onto fiber optical probe and anti-MC-LR antibody. (a) After the HRP labeled secondary antibody was directly introduced into the sample cell and incubated for 300 s, the CL substrate was added; (b) 1.0 μg/mL non-specific anti-BPA antibody instead of anti-MC-LR antibody was added for pre-reaction for 500 s, the CL substrate was added after the HRP labeled secondary antibody was introduced into the cell for incubation; (c) 1.0 μg/mL anti-MC-LR antibody and HRP-labeled secondary antibody were sequentially introduced and pre-reacted for 500 s and 300 s, respectively, the CL substrate was added after washed; (d) The mixture of 1.0 μg/mL anti-MC-LR antibody and 10 μg/L MC-LR and 5.0 μg/mL HRP-labeled secondary antibody were sequentially introduced and pre-reacted for 500 s and 300 s, respectively, the CL substrate was added after washed. The error bars corresponded to the standard deviation of the data points in three repeated experiments (n = 3).

reaction. Part of anti-MC-LR antibodies bound with the MC-LR-OVA immobilized onto the fiber optic immunosensor. Then, 100 μL 5.0 μg/mL secondary antibodies labeled HRP was pumped to the sample cell and reacted with anti-MC-LR antibody for 400 s after washed the excess anti-MC-LR antibody using PBS. To achieve CL detection, 100 μL CL substrate solution was added and the CL signal was detected by PD 3000. Finally, to reuse the fiber optic immunosensor, a regeneration solution (0.5% SDS, pH 1.9) was introduced into the sample cell, and both the primary antibodies and the secondary antibodies was removed. Fig. 3B showed a typical curve of a whole detection process.

In this system, a Si-based photodiode detector PD-3000 instead of PMT is employed for CL detection, whose performance is essential for the whole detection system. Compared to PMT, PD-3000 only needs power supply of 5 V and can tolerate electromagnetic field interference and room light radiation during working without any damage to its performance (Geng et al., 2018). From Fig. 3B, the baseline of FOCB was lower than 50 fW when no CL substrate was added. The sensitivity

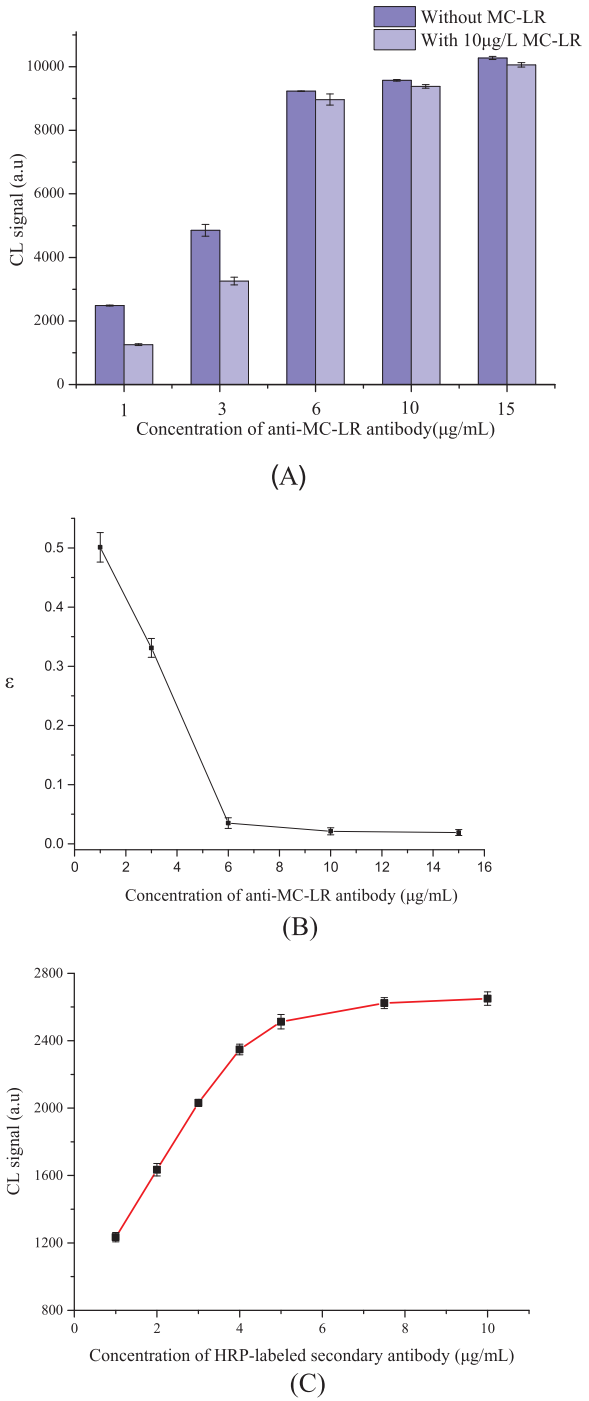

Fig. 4. (A) Optimization of anti-MC-LR antibody concentration (Both pre-reaction and incubation time were 500 s, secondary antibody concentration was 10.0 μg/mL); (B) the sensitivity indexes calculated at different anti-MC-LR antibody concentration. (C) Optimization of HRP-labeled secondary antibody concentration (The concentration of anti-MC-LR antibody was 1.0 μg/mL, both the pre-reaction time and incubation time were 500 s). The error bars corresponded to the standard deviation of the data points in three repeated experiments (n = 3).

of 5 fW for PD 3000 could be obtained based on three times standard deviation of the baseline values (Fig. 3A). When the CL substrate was introduced into the cell, and the CL signal detected by FOCB system was close to 2400 fW, and the signal/noise ratio (SNR) is more than 45. Therefore, the PD-3000 is very suitable for low light intensity detection, especially for CL detection. The relative standard deviation of baseline

signals for several experiments was 6.4% (Inset in Fig. 4A). When the CL substrate was introduced into the cell, the relative standard deviation of CL maximum signal values was 2.6% (Fig. 3A). These indicates that the FOCB system is very stable and the detected signal is uniform enough. It should be noted that the list price of the PD 3000 is only 200 USD/ea, which is less than 1/3 price of a PMT assembly (more than 600 USD/ea) (Fang et al., 2016).

To evaluate the immobilization effectiveness of fiber optic bio-probe and the detected CL signal originated from the specific binding between anti-MC-LR antibody and MC-LR-OVA immobilized onto the biosensing surface, several control experiments were conducted as followings (Fig. 3B). First, 5.0 μg/mL HRP-labeled secondary antibody was directly delivered to the sample cell for 300 s incubation. After washing by PBS, 100 μL CL substrate solution was added. A little CL signal was observed, which indicated no obvious non-specific adsorption of HRP-labeled secondary antibody onto the biosensing surface. Second, 1.0 μg/mL non-specific anti-BPA antibodies were introduced into the biosensing surface. After 500 s incubation and washing, 100 μL 5.0 μg/mL HRP-labeled secondary antibodies was delivered to the sample cell for 300 s. Then, 100 μL CL substrate solution was added. The detected CL signal was as little as that of secondary antibodies that were directly added. However, when 1.0 μg/mL anti-MC-LR antibodies were initially introduced, and 5.0 μg/mL HRP-labeled secondary antibodies were then added. As soon as CL substrate solution was introduced, a strong CL signal was detected. This demonstrated that the HRP-labeled secondary antibodies bound with anti-MC-LR antibodies because of the specific binding between anti-MC-LR antibody and MC-LR-OVA immobilized onto the biosensor surface, and the CL reaction happened because of HRP catalysis. When the sample with a mixture of 1.0 μg/mL anti-MC-LR antibody and 10.0 μg/L MC-LR was introduced, the signal also increased but to a less extent than that without MC-LR. This result showed that part of anti-MC-LR antibody active sites had been occupied by free MC-LR and few antibodies bound to the MC-LR-OVA immobilized onto the biosensor surface, leading to a weaker CL signal. These results confirmed that the observed CL signal originated from the specific reaction between MC-LR-OVA immobilized onto the immunosensor surface and anti-MC-LR antibodies, and the non-specific adsorption of antibody onto the biosensor surface was negligible. All results indicated that the FOCB was suitable to be used for the surface-based CL immunoassay of targets.

3.2. Optimum of detection conditions

Studies were performed during various immunosensing steps to determine the optimal detection conditions, including the concentration of anti-MC-LR antibody and HRP-labeled secondary antibody, pre-reaction time (reaction time between anti-MC-LR antibodies and MC-LR-OVA immobilized on surface of fiber probe) and incubation time (reaction time between secondary antibodies and anti-MC-LR antibodies bound with MC-LR-OVA).

The concentration of anti-MC-LR antibody is essential for the sensitivity of immunosensor. To determine the optimum concentration of anti-MC-LR antibody, a sensitivity index (ε) was introduced as following.

$$\varepsilon = \frac{I_b - I_s}{I_b} \quad (4)$$

where I_b and I_s are the CL intensities of the immunoassay without MC-LR and with MC-LR of a certain concentration, respectively. For the practical application of the immunosensor in the desired dynamic range, the most acceptable concentration of anti-MC-LR antibody was selected according to the following criteria. First, the SNR should be as high as possible (preferably larger than 40) to generate a reasonable CL intensity. Second, the sensitivity index should be as high as possible (favorably over 0.30), because it was conducive to the effective analyte competition in competitive immunoassay, thus resulting in a better sensitivity. Based on these criteria, the experiments were performed with serial dilution of anti-MC-LR antibody (Fig. 4A). Increasing the anti-MC-LR antibody concentration obviously resulted in an increase in CL intensity, especially for that the concentration of anti-MC-LR antibody was less than 6.0 μg/mL. However, the sensitivity indexes decreased with increasing of the anti-MC-LR antibody concentration (Fig. 4B), which demonstrated that low anti-MC-LR antibody concentration was favorable of improving the sensitivity of immunosensor. Although a higher sensitivity index may be obtained using a lower anti-MC-LR antibody concentration, 1.0 μg/mL anti-MC-LR antibody were chosen as the optimal concentration for subsequent MC-LR measurements considering the CL intensity level required.

To investigate the effect of the pre-reaction time between anti-MC-LR antibody and MC-LR-OVA immobilized onto the immunosensor surface on the CL intensity, various pre-reaction times ranging from 100 s to 600 s were tested. Fig. S2 showed that the CL intensity rapidly increased with increasing pre-reaction time when the pre-reaction time was less than 500 s, then slowly increased and reached a plateau. Considering the CL intensity level required and the goal of reducing the assay length duration, the CL intensity of 500 s which is about 95% of a maximum value is regarded as an effective result.

The effect of the concentration of HRP labeled secondary antibody on the CL intensity was also studied. Fig. 4C showed that the CL intensity linearly increased with increasing the concentration of secondary antibody when its concentration was less than 5.0 μg/mL. Over this concentration, the CL intensity increased only a very little and reached a plateau. Because the higher secondary antibody concentration resulted in more reagent consumption and non-specific adsorption, 5.0 μg/mL secondary antibody was selected as the optimal concentration. Then, the effect of the incubation time of secondary antibody was also investigated. Fig. S3 showed that the CL intensity rapidly increased with increasing incubation reaction time when the incubation reaction time was less than 400 s. Considering the CL intensity level required and the goal of reducing the assay length duration, the CL intensity of 400 s which almost reached a maximum value is regarded as the effective result.

3.3. Dose-response curve of MC-LR

Once the general assay parameters had been optimized, quantification analysis of MC-LR was carried out with indirect competitive sandwich-like immunoassay principle based on HRP catalyzed CL reaction. Fig. 5A showed the typical CL signal traces for MC-LR detection using the FOCB. The introduction of MC-LR with increasing concentrations induced the proportional decrements of the CL intensity. The dose-response curve for MC-LR detection was normalized by expressing the CL intensity of each standard point as the ratio to the CL intensity of the blank sample containing no MC-LR (Fig. 5B), and plotted against the logarithm of the concentration of MC-LR using a four-parameter logistic equation (Supporting information). The error bars in the Fig. 5B corresponded to the standard deviation of the data points in triplicate experiments, and all of them were less than 5%, indicating that the good stability of FOCB for detecting MC-LR.

From Fig. 5B, the limit of detection (LOD) for MC-LR determined using three times standard deviation of the mean blank values were 0.03 μg/L. The linear response of MC-LR, considering between 10% and 90% of inhibition, ranged from 0.23 μg/L to 190 μg/L (Tschmelak et al., 2006). This LOD was by far less than the guideline value of 1.0 μg/L in drinking water imposed by WHO (WHO, 2004). The high sensitivity of the FOCB for MC-LR detection was highly comparable to other immunoassay methods of MC-LR (Table S1). Several reasons are response for this. First, the CL-based detection methods have a high sensitivity because of their ultra-low background noise. Second, the high sensitivity and SNR of FOCB system benefit for improving the sensitivity of immunosensor. Third, increasing the amount of the biorecognition molecules through modifying them on both the probe distal end and the

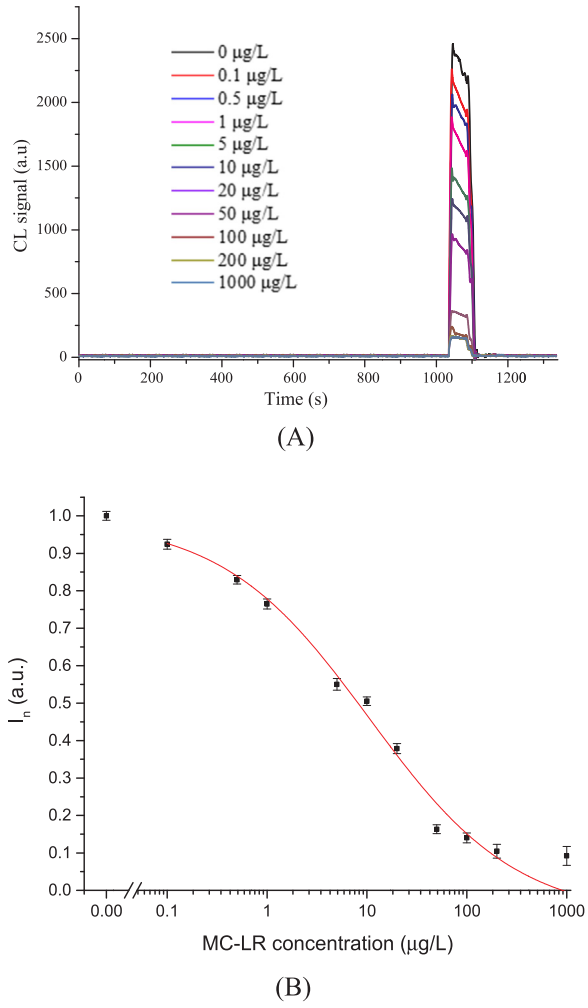

Fig. 5. (A) Typical signal curves of MC-LR detection using FOCB system; (B) dose-response curve of MC-LR detection (1.0 μg/mL anti-MC-LR antibody, 5.0 μg/mL HRP-labeled secondary antibody, pre-reaction and incubation time were 500 s and 300 s, respectively). The error bars corresponded to the standard deviation of the data points in three repeated experiments (n = 3).

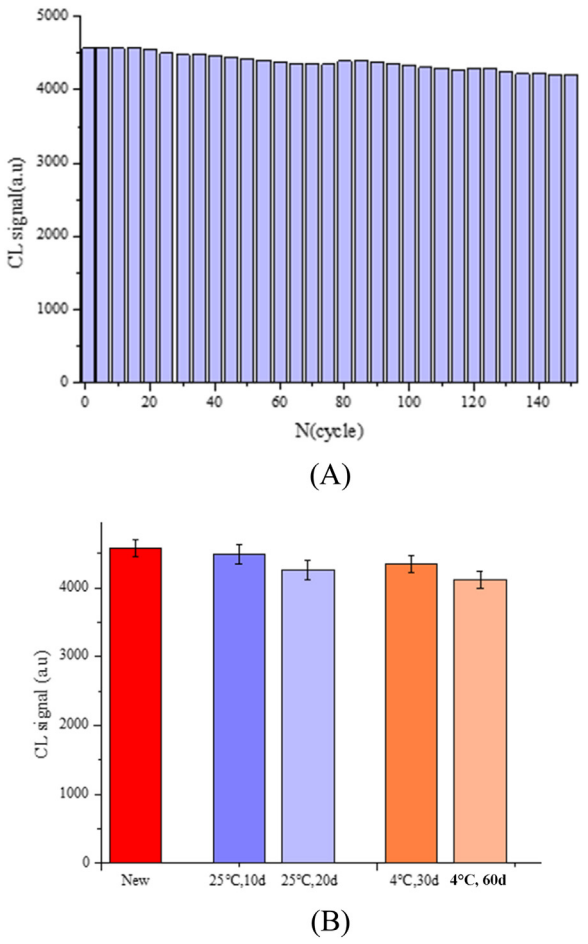

Fig. 6. (A) Maximum signal for immunoreaction of the FOCB in multiple cycles when in the absence of MC-LR; (B) the storage stability of biosensor at different conditions. Detection conditions: 3 μg/mL anti-MC-LR antibody, 10 μg/mL HRP labeled secondary antibody, 500 s pre-reaction time, 400 s incubation time.

cylindrical surface of fiber optical probe could decrease the concentration of anti-MC-LR to obtain the same CL intensity, and lower anti-MC-LR concentration had higher sensitivity index. Fourth, the surface chemistry of the MC-LR-OVA conjugates on the immunosensor surface can keep their high activity toward anti-MC-LR antibodies as well as reduce the non-specific adsorption as previous described (Long et al., 2009). Meanwhile, the FOCB system also manifested its other advantages, such as simple system structure and portability, free magnetic beads and PMT, small sample volume (~ 40.0 μL), and high reusability of the fiber optic bio-probe without compromising immunoreactivity for multiple analyses.

3.4. Regeneration and stability of the immunosensor

Compared to the magnetic-bead based CLIA, one of the main advantages of the proposed optic fiber-based CL biosensor can be reused after regeneration, which is important to reduce the variation of different tests, ensure the accurate detection, and decrease the testing cost. Fig. 5A showed that the fiber optic bio-probe could completely regenerated after washed by 0.5% SDS solution (pH = 1.9). The non-covalently bound primary antibody and secondary antibody could be removed and the detected signal curve came back to the baseline. Actually, Fig. 6A demonstrated that the MC-LR-OVA conjugates modified fiber optic bio-probe could retain at least 150 successive assays without significant loss of activity (less than 5% decrease), indicating that the binding properties of biorecognition molecules were not destroyed by regeneration solution. Except for the reusability of the biosensor, its storage stability is also important from the practical application point of view. When the fiber optic bio-probe was stored at room temperature, it retained more than 95% and 90% of its original response after 10 days and 20 days, respectively (Fig. 6B). Meanwhile, when the bio-probe was stored in a refrigerator at 4 °C, the bio-probe retained about 90% of its original response, even after 60 days of storage (Fig. 6B). Therefore, the cost-effectiveness and reliability of the proposed FOCB in terms of MC-LR measurement is highly advantageous compared to the reported magnetic-bead based CLIA.

3.5. Water sample analysis

The evaluation of matrix effects is another important aspect in the assessment of newly developed CL immunoassay methods. Herein, the recovery experiments of MC-LR at different concentrations were carried out with spiked water samples using lab tap water, commercially available bottled water, and Taihu Lake (Jiangsu, China). These water samples were spiked with MC-LR from the MC-LR stock solution at concentrations of 5.0, 10.0, and 20.0 μg/L. Table S2 showed that the recoveries of the spiked samples were in the range of 80–120%, and the relative standard derivations were 9.0%. These results indicated that the proposed method was capable of detecting MC-LR in real water samples, and the interference from real water samples was negligible.

4. Conclusion

A portable and reusable FOCB system was successfully constructed using fiber optic bio-probe as biorecognition element as well as transducer and a highly sensitive PD-3000 as CL detector. The compact design of FOCB with free magnetic beads and PMT allows the system to be portable and suitable for on-site real-time detection of targets, as well as to be cost-effective and easy-to-use. The new and inexpensive PD-3000 is employed for CL detection instead of PMT, which is enough stable and low-cost, and only needs 5 V voltage. The fiber optic bio-probe with high reusability is used as biorecognition element instead of magnetic beads, which not only simplifies detection processes, but also decreases detection cost and increases the detection accuracy. Due to the high performance of PD-3000 and no background light, the LOD of 5 fW was obtained for the FOCB, which allowed it for highly sensitive CL detection.

Sequentially, the FOCB was applied for the CL-based detection of MC-LR in water using hapten-carrier protein conjugates modified fiber optic bio-probe as biorecognition element. Under optimal conditions, the ultrasensitive detection of MC-LR was achieved, and its LOD is 0.03 μg/L, which is comparable with that of most reported immunoassay methods. Several characteristics of the proposed was demonstrated. First, the robustness of the hapten–carrier protein-modified immunosensor surface allowed multiple MC-LR immunoassays without any significant loss of performance, which ensured accurate and cost-effective detection results. Second, the HRP-labeled secondary antibody instead of HRP-labeled primary antibody was applied for the CL immunoassay of pollutants; hence, the FOCB system is as versatile as CLIA and thus has a good commercial prospect. Third, based on the sandwich-like immunoassay mechanism, the primary antibody of the MC-LR maintained its high activity and its original state due to the absence of modification; thus, the biosensor performance (e.g. sensitivity) was improved. In addition, the FOCB in spiked water samples showed good recovery, precision, and accuracy. All of these results demonstrated that the FOCB could provide a significantly enhanced sensitivity, cost-effective, small sample volume, portability, and automatic operation for the detection of MC-LR well beyond that possible with conventional techniques. The proposed FOCB system can be readily extended toward the on-site real-time sensitive monitoring of other targets in the field of environment, food, and medical diagnosis.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.08.062.

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