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ORIGINAL PAPER

Reusable optofluidic point-of-care testing platform with lyophilized specific antibody for fluorescence detection of cholylglycine in serum

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Abstract

A reusable optofluidic point-of-care testing platform (OPOCT) was successfully constructed through integrating evanescent wave fluorescence technology into an all-fiber-based optofluidic system. The compact design of the OPOCT allows it to be portable and suitable for on-site sensitive determination of biomarkers in serum without complicated and costly procedures. The sensitivity of 90.9 pM for antibody determination is observed thanks to the high transmission efficiency of excitation light and fluorescence in the OPOCT. The affinity constant between cholylglycine (CG) and anti-CG antibody was quantified using this platform based on the proposed theory. Using the lyophilized fluorescence-labeled specific antibody and reusable fiber optic biosensor, the OPOCT is applied to the one-step sensitive determination of CG in serum, which eliminates the dearth associated with liquid reagent handling, disposable biosensors, and user intervention. A limit of detection of 0.025 μg/mL for CG is obtained, which is far more than adequate for meeting diagnostic requirements. The matrix effect of serum samples on the evanescent wave–based optofluidic biosensor can be effectively reduced by simple dilution of serum samples. The performance of the OPOCT also compared favorably with that of a commercial turbidimetric inhibition immunoassay through analyzing multiple serum samples. This platform is ready to expand to measure any other biomarker by using its specific antibody. The simplicity, sensitivity, cost-effectiveness, and robustness of the OPOCT enable the early diagnosis of disease and making a timely clinical decision.

Keywords Cholylglycine · Optofluidic · Evanescent wave fluorescence · Immunoassay · Point-of-testing

Introduction

Cholylglycine (CG), one of the main components of bile acid, is a highly sensitive and specific biomarker to diagnose liver abnormalities [1, 2]. The normal metabolic pathway of CG is enterohepatic circulation [2, 3]. However, abnormal liver cells cannot effectively absorb CG, thus resulting in an increase in serum CG concentration (10~100-fold or higher) [1, 3, 4]. Studies have confirmed that hepatocellular carcinoma (HCC) is associated with the upregulation of the CG [3, 5]. The CG is also regarded as a sensitive indicator of the quantitative evaluation of the fetus condition in the intrahepatic cholestasis of pregnancy (ICP) [1, 6]. The ICP has a great influence on the synthesis of erythropoietin in newborn fetuses, thus inducing the decrease of erythropoietin levels that may lead to fetal hypoxia. Although traditional analytical methods, such as mass spectrometry and enzyme-linked immunosorbent assay, are sensitive and accurate, they require expensive instrument and complicated pretreatment process with time-to-results in the range of several hours or longer [5, 7, 8]. To achieve earlier disease diagnosis and timely patient treatment, developing a rapid, sensitive, easy-to-use platform for the quantitation of the CG in serum with minimal sample preparation is of great importance.

A point-of-care testing device (POCT), in vitro diagnostic test that can provide the result by non-medical staff at home or other places, is a great solution [9]. The integrated biosensor-optofluidic platform, a synergistic combination of biosensor,

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photonics, and microfluidics, is one of the most potential technologies for POCT [9, 10]. Biosensors are ideal alternative analytical tools for the rapid measurement of biomarkers because of their high sensitivity, rapidity, specificity, and easy operation [11, 12]. Optofluidics, integrating photonics and microfluidics, offers unique characteristics such as minimized reagents consumption, shorter reaction times, increased automation, and cost-effectiveness, and is well suited for biological analysis [13, 14]. However, current optofluidic systems require sophisticated coordination of bulky optical components and their rigorous optical design and alignment [13, 15]. These increase the cost of the POCTs and require the complicated reconfiguration and calibration of the optical detection systems if any optical components dislocate. Furthermore, an optical biosensor transducer could not be monolithically integrated with an active microfluidic chip. These limit them to miniaturize and build a portable POCT device. More importantly, most POCT devices use disposable biosensor, which may lead to high cost and inaccurate results because of poor batch-to-batch repeatability [9, 10, 16].

To address these limitations, a novel optofluidic POCT platform (OPOCT) has at the first time been developed through integrating evanescent wave fluorescence technology and all-fiber optofluidic system. In this platform, instead of using numerous optical separated elements, a simple optical module, named as a Y-shaped directional fiber optic coupler (Y-FOC), was constructed for the transmission of excitation light and collection and transmission of evanescent wave fluorescence. The use of the Y-FOC allows the OPOCT to miniaturize and be portable because of its small volume and no requirement of rigorous optical alignment. To further simplify the whole system, a Si-based photodetector with small size, low price, and low power consumption is used for the sensitive detection of weak fluorescence. The combination tapered fiber optic biosensor modified by the coating antigen (CG-BSA) is regarded as a biorecognition element and optical transducer. The evanescent wave generates on the waveguide surface when the excitation light transmits through fiber optic biosensor via total internal reflection (TIR). The evanescent wave excites the fluorescence-labeled anti-CG antibodies bound to the biosensor surface because of its limited penetrated depth (typically < 100 nm) [17]. Part of fluorescence couples back into the fiber biosensor and is detected by a photodiode detector. Biomolecular binding kinetics provides qualitative or quantitative information that can be essential for the advancement of medical diagnosis, drug discovery, and biosensor development [18]. The affinity constant between CG and anti-CG antibodies is determined based on the proposed theory. Using the OPOCT and lyophilized anti-CG antibody, the one-step on-site determination of the CG in serum is achieved. The detection results and the alarm signals may directly send to the health management center through a wireless communication module in the OPOCT, which benefits for the healthy management of disease, especially for HCC and ICP patients.

Experiment section

Reagents and materials

Bovine serum albumin (BSA), 3-mercaptopropyltrimethoxysilane (MTS), N-(4-maleimidobutyryloxy) succinimide (GMBS) were purchased from Sigma-Aldrich (Steinheim, Germany). All other reagents, unless specified, were supplied by Beijing Chemical Agents (Beijing, China), which were all of analytical grade and used without further purification. Deionized water was used throughout the experiment. The phosphate-buffered saline solution (PBS, 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 the antibody dilution buffer (1.0 g BSA, 0.02 g NaN_3, and 100 mL 0.01-mol/L PBS) were prepared, respectively. The sodium dodecyl sulfate (SDS, 0.5%, pH = 1.9) was applied to regenerate the biosensor.

The monoclonal anti-CG antibody produced male BALB/C mice (6 weeks old) and CG-BSA were prepared by our research group. Anti-deoxynivalenol antibody (anti-DON antibody) and anti-enrofloxacin antibody (anti-ENR antibody) were purchased from Shijiazhuang Zhiwei Biological Technology Co. (Hebei, China). All antibodies were labeled using dye Cy 5.5 and stored at − 20 °C. An aliquot of the Cy5.5-anti-CG antibody was lyophilized in a 0.5-mL centrifugal tube. The CG stock solution and various standard solutions were prepared using the PBS and stored at 4 °C.

Apparatus: the OPOCT

Figure 1 a schematically illustrates the setup of the OPOCT device that involves optical module, fluidics module, electronic control module, and data treatment module. The design of the OPOCT is in detail described in Supporting information. In this system, the single-mode fiber optic of the Y-FOC induces the excitation light from 635-nm laser diodes into the combined taper fiber optic biosensor, which is embedded into a microfluidic cell. The fluorescence excited by the evanescent wave is collected by the multimode fiber optic of the Y-FOC and real-time detected by a Si-based photodetector after filtered using a bandpass filter. Various solutions, including buffer, samples, and regeneration solution, are sequentially introduced into the microfluidic cell by the fluidics module. A microcontroller with a self-developed program is used to control valve, pump, and data acquisition and processing. A wireless communication module is integrated into the

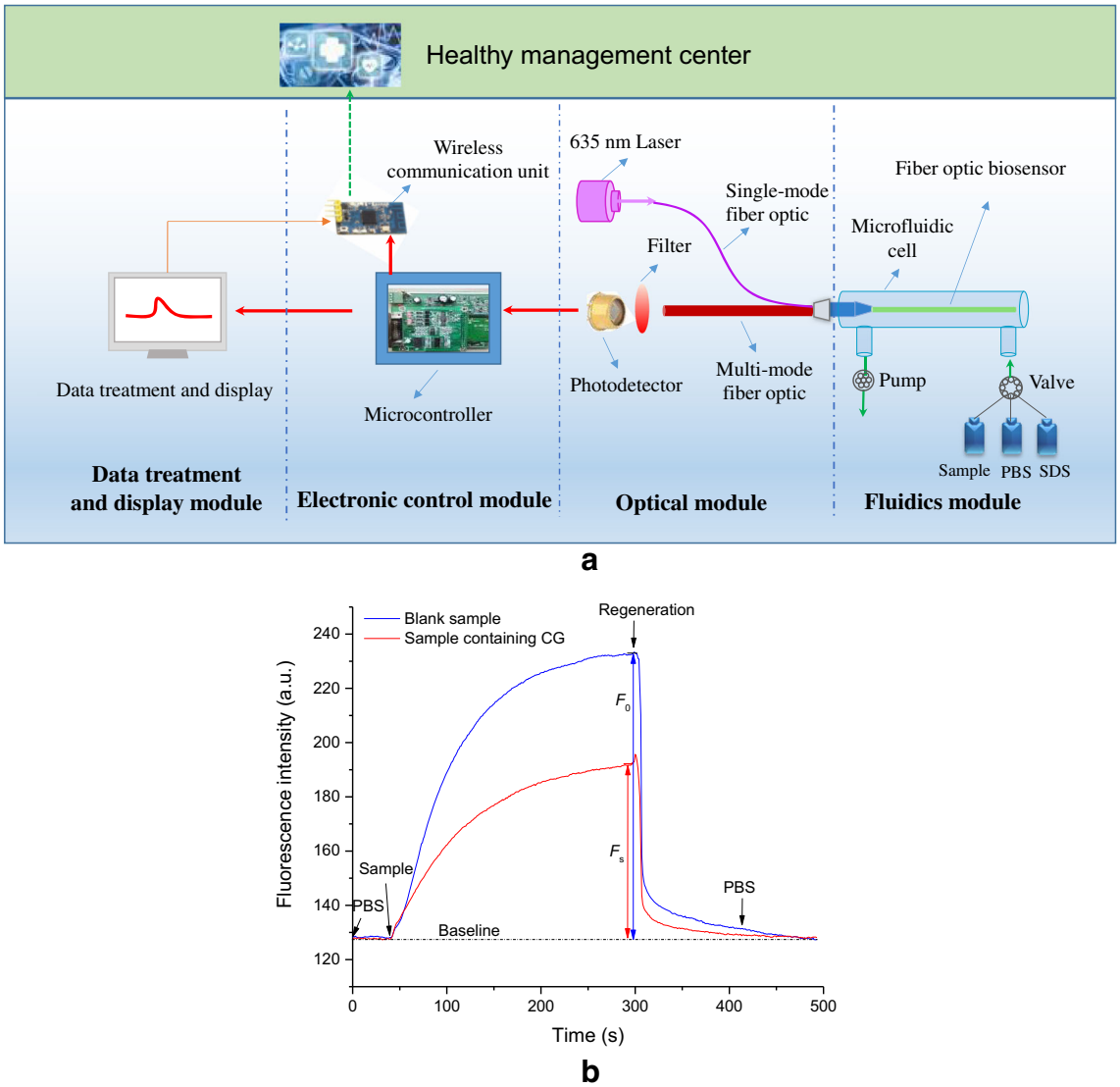


Fig. 1 Scheme of the OPOCT and data treatment. **a** The OPOCT consists of optical module, fluidics module, electronic control module, and data treatment module. **b** The typical fluorescence signal traces detected by the OPOCT. The Cy5.5-anti-CG concentration is 0.125 g/mL, and the CG concentration is 0.25 μg/mL

OPOCT, and the detection results and the alarm signals are directly sent to the health management center.

A mini-computer is employed for user control, data treatment, and result display. The dynamic fluorescence signal trace (Fig. 1b) is real-time demonstrated in the user surface, which reveals the binding reaction kinetics between antigen and antibody. The net fluorescence intensity of the sample is calculated according to Eqs. 1 and 2.

$$F_s = F_{sample} - F_{baseline} \quad (1)$$

$$F_0 = F_{blank} - F_{baseline} \quad (2)$$

where F_0 and F_{sample} are the detected fluorescence intensity of the blank sample and sample containing CG, respectively. $F_{baseline}$ is the baseline value of the OPOCT.

For CG measurement, the normalized value is calculated as Eq. (3).

$$I = \frac{F_s}{F_0} \quad (3)$$

Functionalization of fiber optic biosensor

A combination of tapered fiber optic biosensor is produced using HF acid tube etching method according to our previous report [19]. The total length of fiber optic (600 μm, NA = 0.22) and the effective length of the sensing region are

5.5 cm and 3.0 cm, respectively. The diameter of the sensing region after etching is 220 μm, which allows the biosensor to embrace better excitation efficiency of evanescent wave and collection efficiency of evanescent wave fluorescence [19, 20]. To functionalize a fiber optic biosensor, the hydroxyl groups on the surface of the sensing region were initially activated by a piranha solution (H_2SO_4/H_2O_2, 3:1). Then, the fiber optic biosensor was immersed in a 2% (v/v) MTS solution in ethanol for 2 h to introduce thiol groups onto the silica surface. After washing with ethanol 3 times and drying under N_2 flow, the fiber optic biosensor was immersed in a GMBS solution (0.1 mM, pH 7.0) for 1.5 h. Then, the fiber optic biosensor was placed into the CG-BSA solution, and the free amine groups of CG-BSA covalently bound with the functional groups of GMBS. After washing with PBS, a 2 mg/mL solution of OVA was used to block the residue sites of the fiber optic biosensor for 2 h to eliminate the nonspecific adsorption. After washing with PBS, the fiber optic biosensor was kept at 4 °C for the following experiments.

CG analysis in real serum samples

To demonstrate the application potential, the real serum samples are determined using the OPOCT. The lyophilized Cy5.5-anti-CG antibodies are rehydrated to detect CG in real serum samples. After diluting 20 times, 60 μL real serum sample is directly added into the lyophilized tube containing Cy5.5-anti-CG antibodies with a final antibody concentration of 0.125 μg/mL. The mixture pre-reacts for 4 min and is then introduced into the microfluidic cell by the negative pressure originated from the peristaltic pump. The real-time fluorescence signal is detected by the OPOCT. Finally, the biosensor surface can be regenerated using the SDS solution for 120 s. After washing by PBS solution for 90 s, the biosensor can be used for the next test.

Results and discussion

Fabrication and characterization of the OPOCT

The compact optical module is a key unit to construct a portable optical POCT device [9, 10, 16]. In the optical module of the OPOCT, the Y-FOC structure is applied for the transmission of excitation light and collection and transmission of evanescent wave fluorescence. The fiber optic biosensor modified by CG-BSA is regarded as a biorecognition element and transducer. The excitation light enters the fiber optic biosensor through the single-mode fiber optic of the Y-FOC and transmits in it via total internal reflection. The evanescent wave excites the Cy5.5-anti-CG antibodies bound with CG-BSA on the biosensor surface. The fluorescence coupled back into the fiber optic biosensor is collected by the multimode fiber optic of the Y-FOC and is real-time detected by a Si-based photodetector with high sensitivity after filtered by a bandpass filter. To verify the sensitivity of the OPOCT, various concentrations (0.06, 0.125, 0.25, 0.5, and 1.0 μg/mL) of the Cy5.5-anti-CG antibody were introduced into the microfluidic cell, the detection fluorescence intensity increased rapidly, and an equilibrium state was then reached (Fig. 2a). The fluorescence intensity linearly increased with increasing antibody concentration due to more antibodies bound to the biosensor surface (Fig. 2b). The linear coefficient was 0.982 in the experimental concentration range, and the relative standard deviation (RSD) of each concentration point was less than 2.3%, indicating the good stability of OPOCT. As shown in Fig. 2a, when 0.06 μg/mL anti-CG antibody was pumped over the biosensor surface, an obvious fluorescence signal increase can be observed. The limit of detection (LOD) of the OPOCT for Cy5.5-anti-CG antibody was 0.015 μg/mL (or 90.9 pM) using 3σ of the mean blank values according to IUPAC recommendations.

To further characterize the performance of CG-BSA-modified fiber optic biosensor, several experiments were conducted as follows. First, when 0.125 μg/mL Cy5.5-anti-CG antibody is introduced, the fluorescent signal increases rapidly over time due to the anti-CG antibody specifically bound with the CG-BSA immobilized on the biosensor surface (Fig. 1b). However, when a mixture of 0.125 μg/mL anti-CG antibody and 0.25 μg/mL CG is introduced, the fluorescence signal also increases but to a less extent than that without CG, which contributes to part binding sites of anti-CG antibodies are occupied by free CG in solution (Fig. 1b). Second, the other antibodies (e.g., Cy5.5-anti-ENR antibody and Cy5.5-anti-DON antibody) are added, and few fluorescence intensities are observed even when their concentrations (1.0 μg/mL) are higher than those of anti-CG antibody (Fig. 2b). These results demonstrate that the CG-BSA conjugates are successfully modified onto the biosensor surface, and few nonspecific adsorptions occurred and few free fluorescence molecules are excited. The fluorescence signals detected by the OPOCT originate from the binding reaction between the Cy5.5-anti-CG antibody and CG-BSA on the biosensor surface. Third, the reusability of the CG-BSA-modified fiber optic biosensor is verified using SDS solution as a regeneration reagent. As shown in Figs. 1b and 2a, the fluorescence signal trace rapidly decreases when the regeneration solution (0.5% SDS solution) is introduced into the cell [21]. After washing by PBS, the signal trace returns to the baseline, indicating that the Cy5.5-anti-CG antibodies bound to the biosensor surface are completely removed. Actually, the CG-BSA-modified fiber optic biosensor can be reused more than 100 times without significant activity loss (Figure S2). Compared with that of the disposable biosensors, the reusability of fiber optic biosensor in the OPOCT not only avoids the batch-to-batch difference

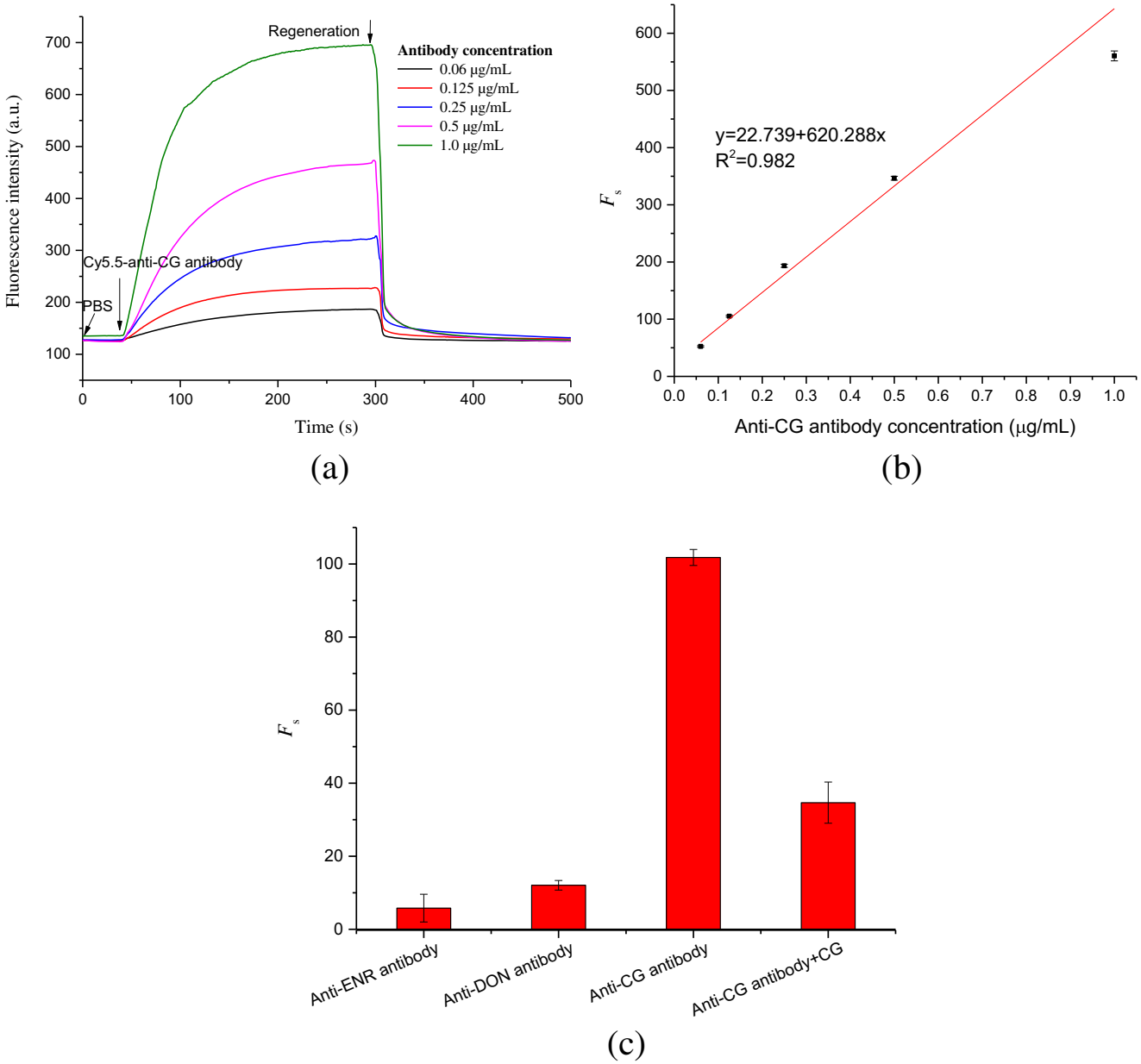


Fig. 2 Characterization of the OPOCT. **a** The representative fluorescence signal traces detected by the OPOCT at various concentrations of Cy5.5-anti-CG antibody (from 0.06 to 1.0 μg/mL). **b** The linear relationship between antibody concentration and fluorescence intensity. **c** Assessing the modification effectiveness of the fiber optic biosensor and its specific binding performance with anti-MC-LR antibody. The concentration of Cy5.5-anti-CG antibody and CG is 0.125 μg/mL and 0.25 μg/mL, respectively. The concentration of both Cy5.5-anti-DON antibody and Cy5.5-anti-ENR antibody is 1.0 μg/mL. The error bars corresponded to the standard deviation of the data points in three repeated experiments ($n = 3$)

and improves the accuracy of detection results, but also decreases the detection cost. All of these confirmed that the OPOCT is suitable to be used for CG immunoassay with high sensitivity and reusability.

Binding kinetic analysis between CG and anti-CG antibody

Immunosensor is one of the most popular and efficient technologies to measure the binding kinetics between biomolecules [18]. Herein, the OPOCT is used to investigate the affinity constant between CG and anti-CG antibody. The experimental results demonstrated that the fluorescence signal originated from the binding reaction between anti-CG antibody and CG-BSA immobilized on the biosensor surface because of the limited penetrated depth of evanescent wave. This allows the measurement of the binding kinetics between CG and anti-CG antibody. To determine the affinity constant, the following assumptions should be satisfied: (a) the interaction of antigen/antibody belongs to first-order kinetics; (b) the

interactions between binding sites do not exist; (c) the number of antigen-binding sites is limited; (d) the surface density of the antigen is lower than the amount of the antibody in solution; (e) the dissociation constant is much lower than the association rate constant. According to these, the kinetic equation between CG and anti-CG antibody can be written as

$$\frac{\partial[AB]}{\partial t} = k_a[A]_0[B]_0 - \left(k_a[A]_0 + k_d\right)[AB] \quad (4)$$

where $[AB]$ is the time-dependent concentration of the CG/anti-CG antibody complex. $[A]_0$ is the initial concentration of the Cy5.5-anti-CG antibody, and $[B]_0$ is the CG concentration averaged over the biosensor surface. k_a is the association rate constant, describing the formation rate of the CG/anti-CG antibody complex, and k_d is the dissociation rate constant involving the stability of the CG/anti-CG antibody complex. Under quasi-steady-state approximation of the reaction, $d[AB]/dt = 0$, Eq. 4 can be expressed as follows:

$$[AB]_{eq} = \left(\frac{K_D}{[A]_0} + 1\right)^{-1}[B]_0 \quad (5)$$

where $[AB]_{eq}$ is the concentration of CG/anti-CG antibody complex at quasi-steady-state. $K_D = k_d/k_a$ is the equilibrium dissociation constant. Because $[AB]$ is proportional to the fluorescence intensity detected by the OPOCT, Eq. 5 can be written as

$$\frac{1}{[F]_{eq}} = \frac{K_D}{[A]_0}\frac{1}{[F]_{max}} + \frac{1}{[F]_{max}} \quad (6)$$

where $[F]_{eq}$ is the fluorescence intensity at quasi-steady-state for a certain concentration of the Cy5.5-anti-CG antibody; $[F]_{max}$ is the maximum fluorescence intensity when all binding sites of the biosensor surface are occupied by the Cy5.5-anti-CG antibody. Eq 6 presents a linear relationship between $1/[F]_{eq}$ and $1/[A]_0$. The relationship between anti-CG antibody concentration and fluorescence intensity is shown in Fig. 2 a and b. Plots summarizing the concentration-dependent anti-CG antibody binding to CG-BSA exhibit characteristic linear response (Fig. 2b), indicating that the immunoreaction satisfies the first-order kinetics. Therefore, K_D is calculated as 25.5 nM by using the slope of the linearity between $1/[AB]_{eq}$ and $1/[A]_0$ (Fig. 3), as described in Eq. 6 given above. This demonstrates that the OPOCT can serve as a simple and potentially quantitative tool for a binding reaction between biomolecules.

Dose-response curves of CG using as-prepared and lyophilized anti-CG antibody

Based on the indirect competitive immunoassay mechanism, quantification detection of CG is initially carried out using an as-prepared Cy5.5-anti-CG antibody (Fig. 4a, left). First, 30 μL of the sample containing various concentrations of CG is mixed with 30 μL of 0.125 μg/mL anti-CG antibody that is prepared from the antibody stock solution. During the 4-min pre-reaction process, some of the anti-CG antibodies bind with CG, and the binding sites of the antibodies are proportionally occupied according to the CG concentration in the samples. Second, the mixture is introduced into the microfluidic cell, and the antibodies with free binding sites bind to the CG-BSA on the biosensor surface. The fluorescence intensity detected by the OPOCT increases over time and reaches a plateau (Fig. 4b). Finally, 0.5% SDS solution (pH 1.9) is injected into the cell to reuse the biosensor. The fluorescence signal trace decreases and comes back to the baseline after washed by PBS, and the whole detection is less than 15 min. As shown in Fig. 4b, the higher concentration of CG in the samples leads to the more antibodies to bind with them, which prevents the antibodies to bind with CG-BSA on the biosensor surface, and induces the proportional decrease of the fluorescence intensity. Using a four-parameter logistic equation (Supporting information), the dose-response curve of CG is plotted against its concentration logarithm (Fig. 4d). The linear response of CG ranges from 0.063 to 0.98 μg/mL. The LOD for CG determined using three times standard deviation of the mean blank values is 0.021 μg/mL, which is far lower than the normal level (0.4 to 2.98 μg/mL) of the healthy persons [1, 22].

To explore the potential use of OPOCT in the field, the lyophilized Cy5.5-anti-CG antibodies are rehydrated to detect CG in samples (Fig. 4a, right). Different from the as-prepared antibody, 60 μL samples containing various concentrations of CG can be directly added into the lyophilized tube containing Cy5.5-anti-CG antibodies, and a final antibody concentration is 0.125 μg/mL. After a 4-min pre-reaction, the mixture is introduced into the microfluidic cell to detect CG concentration. As shown in Fig. 4c, the typical fluorescence signal traces using the lyophilized Cy5.5-anti-CG antibodies are similar to those of an as-prepared anti-CG antibody, indicating the activity of the lyophilized antibody does not decline. The dose-response curve is also plotted against the concentration logarithm of CG (Fig. 4d). The LOD of CG is 0.025 μg/mL, and the linear response of CG ranges from 0.071 to 1.00 μg/mL. No statistically significant differences ($P < 0.001$) were observed between the calibration plots obtained using as-prepared or lyophilized antibody. The high sensitivity of the OPOCT for CG detection, which is highly comparable with the LOD of 0.026 μg/mL of the turbidimetric inhibition immunoassay method (TIIA) (Figure S3) and other methods (Table S1), should contribute to the following reasons. First, the high sensitivity and signal-to-noise of the OPOCT system benefit for improving immunoassay sensitivity. Second, the surface chemistry of the CG-BSA conjugates on the biosensor surface can keep their high activity toward anti-CG antibodies

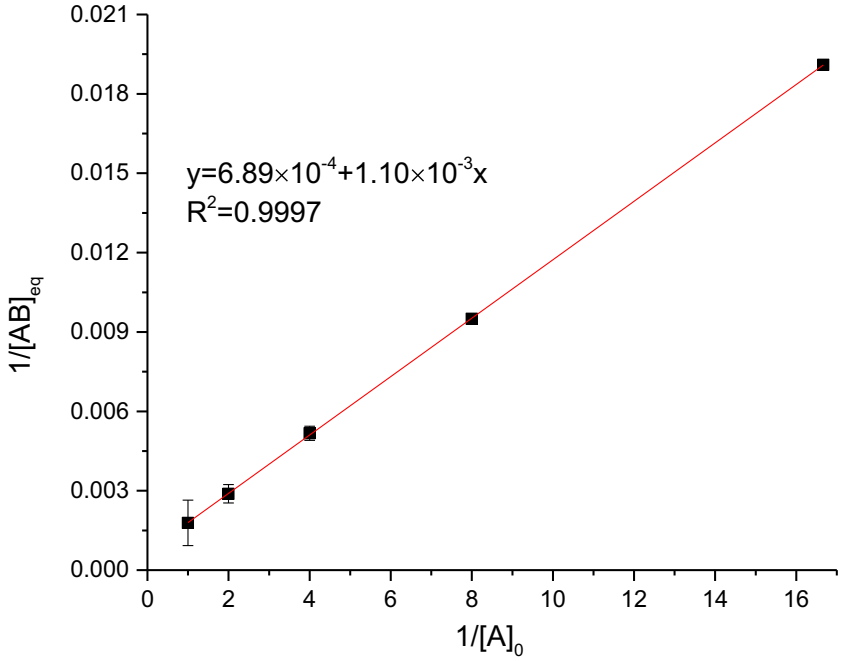


Fig. 3 Dependence of $1/[AB]_{eq}$ against $1/[A]_0$ for the association of Cy5.5-anti-CG antibody on CG-BSA on biosensor surface. The error bars corresponded to the standard deviation of the data points in three repeated experiments ($n = 3$)

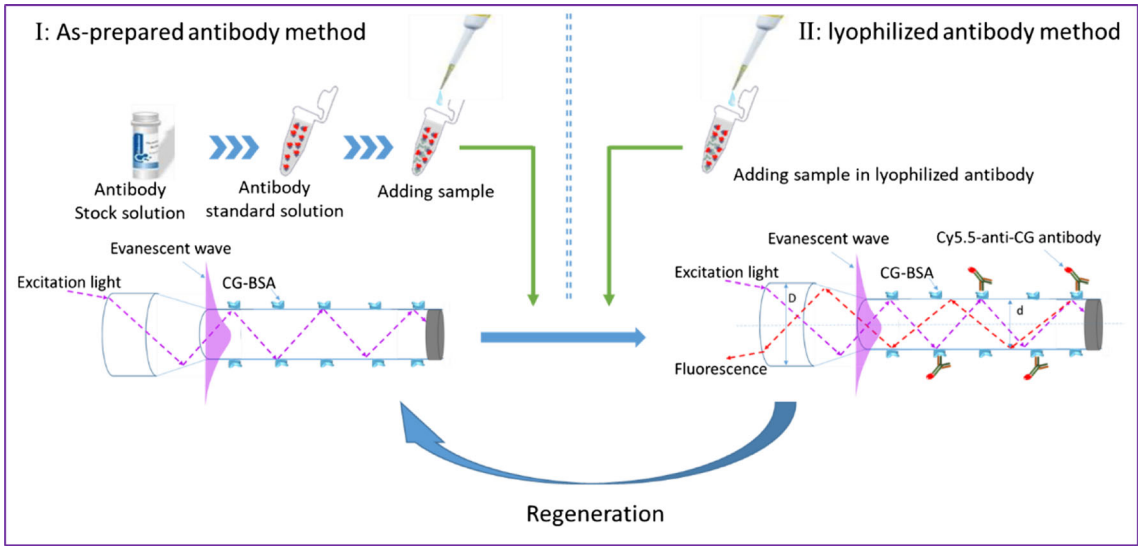
as well as reduce the nonspecific adsorption as previously described [19]. More importantly, the OPOCT can directly detect the sample using the lyophilized antibody, which is not only beneficial for its cold chain independence and long-term storage, but also readily reconstitutes for rapid on-site detection [23].

Matrix effect of serum on the binding reaction between antibody and antigen on biosensor surface

It is essential for directly detecting CG in serum without a complex pretreatment process at POCT settings. However, the matrix complexities of the serum are challenging for accurate analysis of real samples using biosensor because they can affect the interaction between antibody and antigen [8, 24, 25]. Herein, we chose the Cy5.5-anti-ENR antibody and ENR as a model to investigate the matrix effect of serum to avoid interference of the CG in the serum. The ENR-BSA was modified on the fiber optic biosensor surface, which was embedded in the cell to be regarded as a biorecognition element and transducer. The Cy5.5-anti-ENR antibody was added into the PBS, primary serum, 5 times, 10 times, and 20 times diluted serum, respectively, with a final antibody concentration of 0.5 μg/mL. These samples were respectively introduced over the ENR-BSA-modified biosensor surface to detect fluorescence signals. As shown in Fig. 5a, the fluorescence intensity of primary serum is by far lower than that of the PBS solution, which may mainly contribute to the inhibition of the serum matrix on the binding reaction between antibody and antigen as previous reporters. This is further verified using an unmodified fiber optic biosensor to detect the fluorescence intensity of free Cy5.5-anti-ENR antibody in serum and PBS solution (Figure S4), respectively. Interestingly, the fluorescence intensity of the free Cy5.5-anti-ENR antibody in serum is slightly higher than that in PBS solution, indicating that the serum matrix does not affect the fluorescence intensity of Cy5.5-anti-ENR antibody. To reduce the inhibition of the serum matrix, we simply diluted the serum using PBS solution. We found that the detected fluorescence intensity increased with increasing the diluted times of serum (Fig. 5). When the serum is diluted 20 times, the detectable fluorescence intensity is almost the same as that in PBS. Although sample extraction and cleanup procedures are effective ways to eliminate matrix effects in blood samples, they are generally time-consuming and laborious and not suitable for POCT [24, 26, 27]. Dilution of the blank matrix is obviously a valuable method to account for decreasing interference. Therefore, if not a specific statement, all the serum samples are diluted 20 times to test in the following experiments.

Analysis of real serum samples using the OPOCT and comparison with TIIA

To demonstrate the clinical application of the OPOCT, 12 serum samples, including 9 mouse samples and 3 human serum samples, were measured by the OPOCT. As described above, dilution is a simple and effective method to reduce the matrix effect of serum. Therefore, all samples were diluted 20 times before testing. For comparison, these samples were also detected using a commercial TIIA instrument (Supporting information). The CG contents of 9 mouse samples range from 6.34 to 13.11 μg/mL. The CG contents of 3 human serum are



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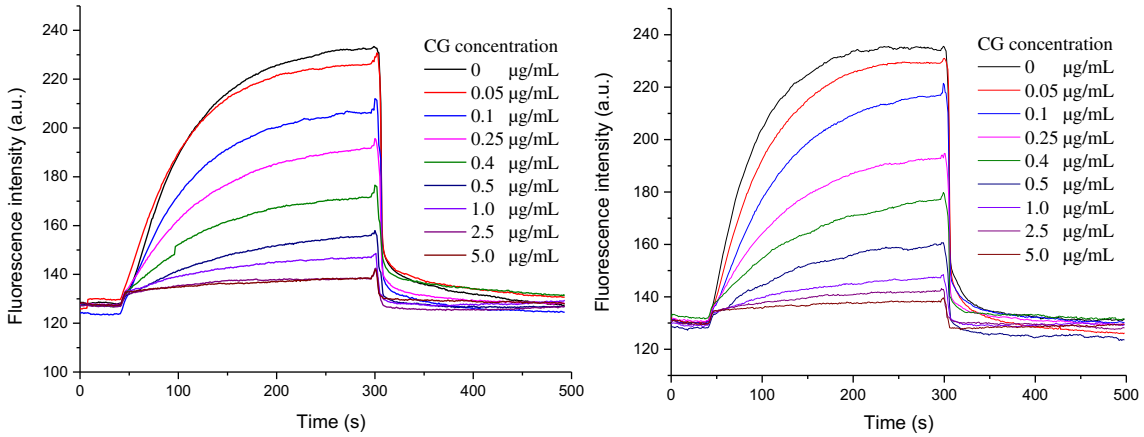

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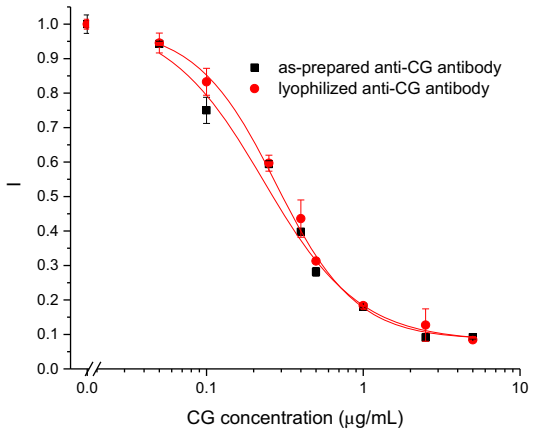

d

Fig. 4 Determination of CG using the OPOCT. **a** The sensing mechanism of CG using as-prepared (left) or lyophilized Cy5.5-anti-CG antibody (right). **b** Typical fluorescence signal curves of CG detection using as-prepared 0.125 μg/mL Cy5.5-anti-CG antibody. **c** Typical fluorescence signal curves of CG detection using the lyophilized Cy5.5-anti-CG antibody with final concentration of 0.125 μg/mL. **d** Dose-response curves of CG detection using as-prepared and lyophilized Cy5.5-anti-CG antibody. The error bars corresponded to the standard deviation of the data points in three repeated experiments ($n = 3$)

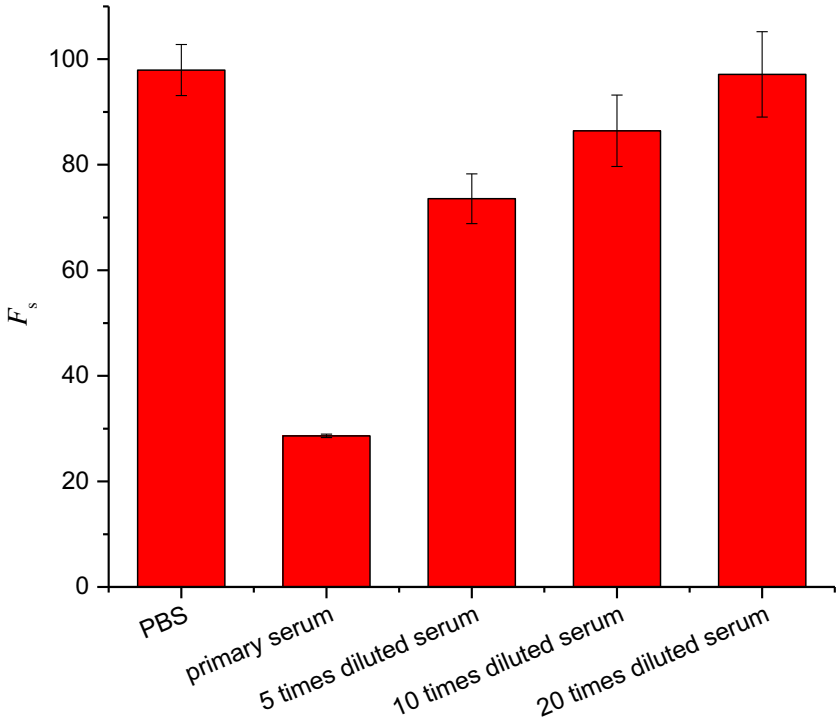

Fig. 5 Matrix effect of serum on the binding reaction between antibody and antigen on biosensor surface. The 0.5 μg/mL Cy5.5-anti-ENR antibody was added into the PBS, primary serum, 5 times, 10 times, and 20 times diluted serum, respectively. The ENR-BSA-modified fiber optic biosensor is used to detect fluorescence signal values. The error bars corresponded to the standard deviation of the data points in three repeated experiments ($n = 3$)

from 3.45 to 6.07 μg/mL, which are higher than the health level. A comparison between the methods yields good correlation results ($y = 0.896x + 0.272$) (Fig. 6 and Table S2), and the correlation coefficient ($R^2 = 0.986$) demonstrates satisfactory agreement between the methods, indicating the adequacy of the OPOCT for accurate CG determination. These results demonstrate the excellent performance of the present OPOCT for the measurement of CG in serum in a clinical setting.

Conclusions

The OPOCT platform was constructed through integrating evanescent wave technology and all-fiber optofluidic system. The compact design of this system allows it to be portable and suitable for one-step on-site sensitive determination of biomarkers in serum samples, as well as be cost-effective and easy to operate. Using the lyophilized Cy5.5-anti-CG

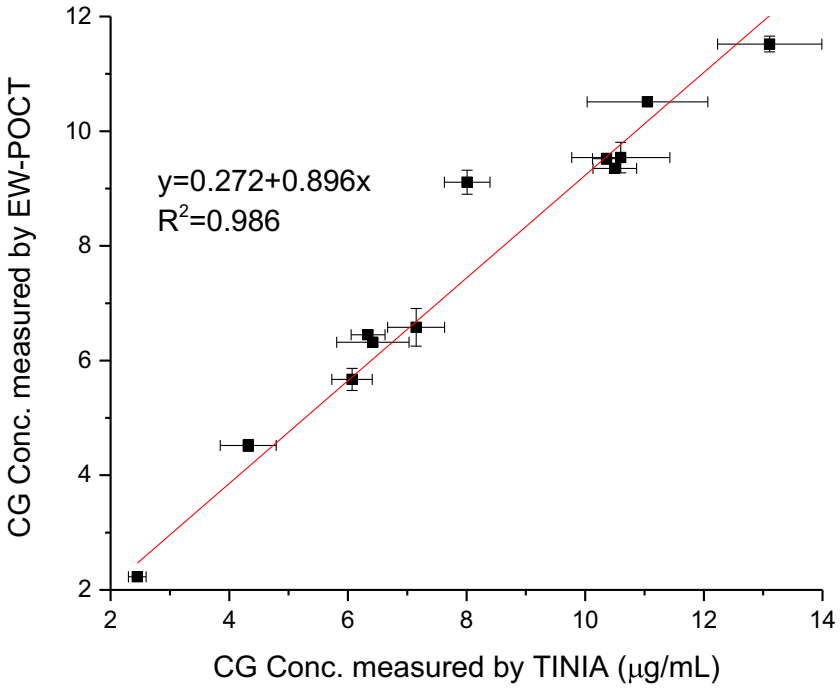

Fig. 6 Comparison of CG levels in 12 serum samples measured by the proposed OPOCT and those measured by TIIA. The error bars corresponded to the standard deviation of the data points in three repeated experiments ($n = 3$)

antibody, this platform gave excellent linearity over the range of 0.071 to 1.00 μg/mL and displayed an LOD of 0.025 μg/mL for CG, which was far more than adequate for meeting diagnostic requirements. The quantitation of affinity constant of the CG/anti-CG antibody was also achieved using the OPOCT based on the proposed theory. The matrix effect of serum samples on the evanescent wave fluorescence biosensor was investigated, which could be effectively reduced by simple dilution of serum samples. The performance of the OPOCT was also comparable with that of a commercial TIIA instrument by analyzing real serum samples.

Several characteristics of the OPOCT were demonstrated as follows. First of all, the Y-FOC system is employed for the transmission of excitation lights and the collection and transmission of evanescent wave fluorescence, which simplifies the whole structure of the OPOCT without the requirement of rigorous optical alignment, as well as improves its sensitivity and portability. Second, compared with the disposable biosensor in traditional POCT, the reusable fiber optic biosensor in the OPOCT allows multiple CG immunoassays without significant activity loss. This can not only ensure accuracy, but also satisfy the cost-effective requirement for a commercial POCT instrument. Third, the use of the lyophilized antibody allows fewer user steps and cold chain independence and provides a longer shelf life for the test, which will promote the clinical application of the POCT instrument. In addition, this platform can readily be adapted to measure any other biomarker by using its specific antibodies. These qualities allow the OPOCT to enable the early diagnosis of disease and making a timely clinical decision, especially in resource-limited settings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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