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Portable evanescent wave fiber biosensor for highly sensitive detection of *Shigella*

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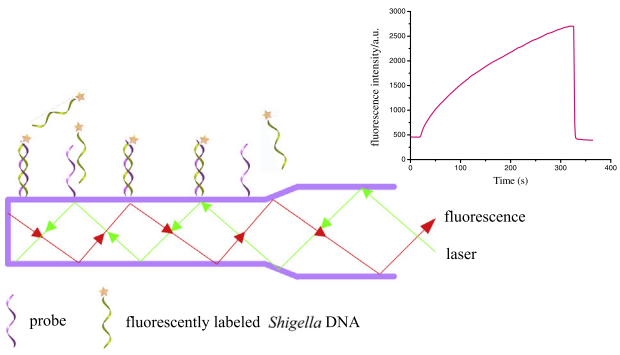
HIGHLIGHTS

- A portable evanescent wave fiber biosensor is developed to achieve the rapid and highly sensitive detection of *Shigella*.
- The sensor probe is home-made and can be used repeatedly.
- The fiber biosensor can be used in high-sensitivity online detection in fields like medical, biological, and environmental.

GRAPHICAL ABSTRACT



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ABSTRACT

A portable evanescent wave fiber biosensor was developed to achieve the rapid and highly sensitive detection of *Shigella*. In this study, a DNA probe was covalently immobilized onto fiber-optic biosensors that can hybridize with a fluorescently labeled complementary DNA. The sensitivity of detection for synthesized oligonucleotides can reach 10^{-10} M. The surface of the sensor can be regenerated with 0.5% sodium dodecyl sulfate solution (pH 1.9) for over 30 times without significant deterioration of performance. The total analysis time for a single sample, including the time for measurement and surface regeneration, was less than 6 min. We employed real-time polymerase chain reaction (PCR) and compared the results of both methods to investigate the actual *Shigella* DNA detection capability of the fiber-optic biosensor. The fiber-optic biosensor could detect as low as 10^2 colony-forming unit/mL *Shigella*. This finding was comparable with that by real-time PCR, which suggests that this method is a potential alternative to existing detection methods.

Introduction

Shigella is a species of enteric bacteria that causes disease in humans and other primates. Most people who are infected with *Shigella* develop various symptoms, such as diarrhea, fever, cramping, vomiting, and other serious complications and illnesses. According to the World Health Organization, the annual number of *Shigella* cases worldwide is estimated to be 164.7 million with 1.1 million deaths, most of which involve children under 5 years old. For adult patients, 10 colony-forming unit (CFU) to 100 CFU of *Shigella* can cause intestinal infections and severe inflammatory responses [1]. Developing countries have a high incidence of dysentery because of the insufficient supply of clean water, poor sanitation, overcrowding, and malnutrition. Thus, the fast and effective detection of *Shigella* is of particular importance.

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Traditional analytical methods for *Shigella* detection include bacterial cultivation, serological methods, and polymerase chain reaction (PCR) [2–9]. However, bacterial cultivation is a labor-intensive and time-consuming process that requires professional skills. Serological methods are simple methods but suffer from low sensitivity and specificity. Despite being more precise than the other methods, PCR requires complex procedures and expensive equipment, thereby preventing online and real-time detection. Thus, a fast, sensitive, and specific method for *Shigella* detection must be urgently developed.

In this paper, we report a novel, highly sensitive evanescent wave fiber biosensor for *Shigella* detection in aqueous solution or food. The sensing time, sensitivity, specificity, and reusability of the biosensor were validated. We also compared the *Shigella* DNA detection sensitivity of the portable fiber-optic biosensor with that of real-time PCR.

Table 1
Oligonucleotides.[a]

Name	Sequence (5′–3′)
IPA probe	Biotin-TTTTTTTTTTTTAGTCTTTCGCTGTTGCTGCTGATGCC
FC TGT	Cy5.5-GGCATCAGCAGCAACAGCGAAAGACT
BPM TGT	Cy5.5-GGCATCAGCACCAACAGCGAAAGACT
NC TGT	Cy5.5-TGGCAGAGCGGGTACTAACATGATT
Forward primer	GGATTCCGTGAACAGGTCGC
Reverse primer	Cy5.5-GATGGACCAGGAGGGTTTTC

[a] TGT = target; FC = fully complementary; NC = non-complementary; BPM = base pair mismatched.

Experimental methods

Materials and reagents

Bovine serum albumin (BSA), (3-aminopropyl)triethoxysilane (APTES), and streptavidin (SA) were purchased from Sigma–Aldrich (Germany). The sequences of all DNA oligonucleotides used in experiments were purchased from Sangon Biotech (Shanghai) Co., Ltd. (China) (Table 1). All solutions were prepared with ultrapure water from a Millipore Milli-Q system. All other salts and reagents were purchased from Sinopharm Chemical Reagent Co.,

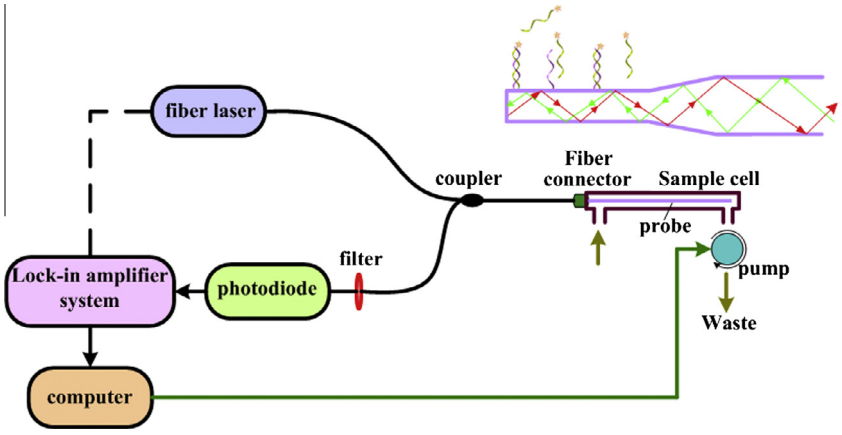

Fig. 1. Schematic of evanescent wave fiber biosensor system.

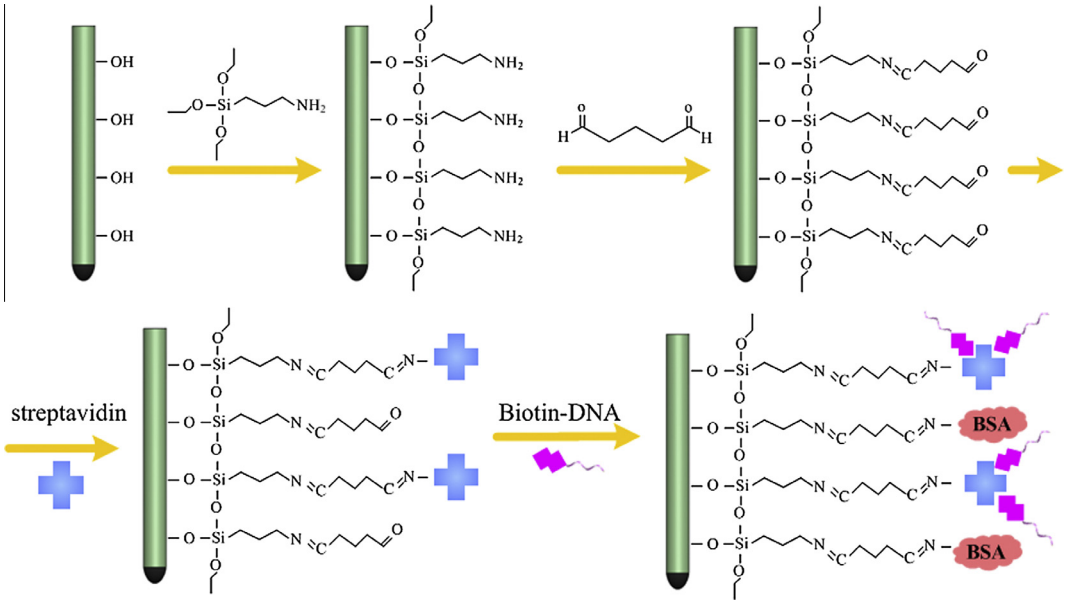

Fig. 2. Schematic of fiber probe modification.

Ltd. (China) unless otherwise specified. All chemicals were of analytical reagent grade.

Bacterial preparation and DNA extraction

Shigella (*Shigella sonnei* CMCCB 51570) used in this experiment was provided by Zhejiang Provincial Center for Disease Control and Prevention. The bacterial concentration was determined using the conventional surface plate counting method. The concentration was then serially diluted to the desired concentrations using PBS. Approximately 1 mL of each concentration of *Shigella* was centrifuged at 10,000*g* for 5 min to pellet the bacterial cells. The pellet was placed in lysis buffer, and the DNA was extracted using the TIANamp Bacteria DNA kit (Tiangen, Beijing, China) according to the manufacturer's protocol.

Instrumentation: all-fiber evanescent wave biosensor

The schematic of the all-fiber evanescent wave biosensor is shown in Fig. 1. The laser beam from a 635 nm pulsed diode laser (BWT, Beijing, China) with pigtail was directly launched into a single-mode fiber of a single-to-multimode fiber coupler (Beijing Glass Research Institute, China), thereby reducing the optical components and removing the need for optical alignment. Then, the excitation light from the laser was coupled to a fiber probe through the fiber connector. The incident light then propagated along the length of the probe through total internal reflection. The evanescent wave generated at the surface of the probe then interacted with the surface-bound fluorescently labeled analyte complexes. This interaction caused the excitation of fluorophores. The collected fluorescence was subsequently filtered using a band-pass filter (FF01-692/40, Semrock, USA) and detected by the photodiode through a lock-in amplifier system. The lock-in amplifier system effectively acted as a narrow band-pass filter, which removed much of the unwanted noise while allowing the signal which was to be measured to pass through. The probe was embedded in a glass flow cell with a flow channel (60 mm length and 2 mm diameter). All reagents were delivered by a flow delivery system operated with a peristaltic pump. The control of the fluid delivery system and data processing were automatically performed by the computer.

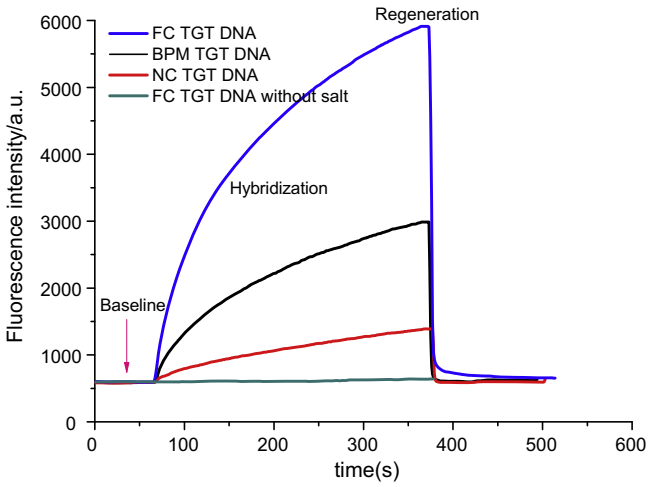

Fig. 3. Typical signal trace observed in the flow of various target DNA solutions on the sensor surface. FC TGT: fully complementary target DNA; BPM TGT: one-base mismatched DNA; NC TGT: non-complementary target DNA.

Fiber probe modification

The probes were made of a step-index silica optical fiber with length of 11 cm and diameter of 600 μm (Chunhui Science and Technology Industrial Co., China). The cladding was removed at 6.5 cm along the distal end to form the sensing region. Removal of cladding, however, resulted in the number of modes mismatch between the cladding and the sensing region of the fiber [10,11]. A fraction of fluorescent signal coupling into the sensing region was lost on entering the cladded fiber. To prevent this loss from occurring, the radius of the fiber's sensing region can be reduced. So, this region was then tapered by immersion into hydrofluoric acid, as in tube etching. The optimum matching radius of the sensing region was 223 μm. The lengths of the tapered section and the sensing region were approximately 0.3 and 6.0 cm, respectively. Combination-tapered fiber probes were immersed in a series of solutions of H_2SO_4/30% H_2O_2 (volume ratio 3:1), 25% NH_4OH/30% H_2O_2/water (volume ratio 1:1:5), and 36% HCl/30% H_2O_2/water (volume ratio 1:1:5). Finally, the probes were sonicated with water and dried using N_2. The modification process of the fiber probe is illustrated in Fig. 2. The cleaned fiber probes were aminated by

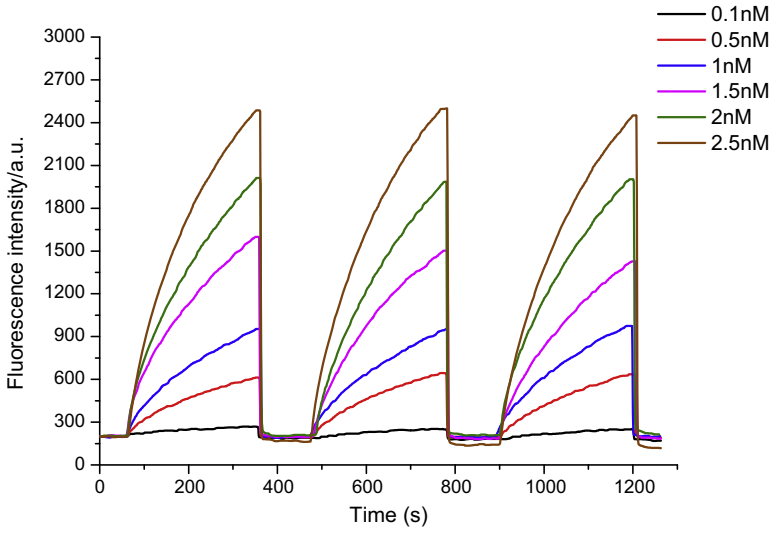

Fig. 4. Signal traces in detections of three consecutive regenerations of different DNA concentrations.

incubation in a solution composed of 12 mL of 95% ethanol, 12 μL of acetic acid, and 150 μL of APTES at room temperature for 30 min. The probes were then sonicated in 95% ethanol for 4 min and dried using N_2. The aminated fiber probes were incubated in 10% glutaraldehyde solution for 1 h and rinsed. Then, the aldehyde fiber probes were transferred to 0.05 mg/mL SA for 3 h and washed with PBS plus 0.05% Tween 20 (PBST). The SA fibers were placed in 1 μM biotin-labeled DNA probe solution for 1 h. After rinsing with PBST, the fiber probes were dipped in 1% BSA solution for 1 h to block the non-specific absorption sites.

Sensor detection procedure

The Cy5.5-labeled target DNA in hybridization buffer (20 mM Tris–HCl, pH 8.0, 0.5 M $MgCl_2$) was pumped into a 200 μL reaction cell and allowed to bind to the biotin-labeled DNA probe at room temperature for 5 min. The real-time fluorescence signal was collected. The fiber probe surface was then regenerated with 0.5% sodium dodecyl sulfate (SDS) solution (pH 1.9) for 1 min and washed with PBST.

The PCR products were detected by the fiber biosensor following the same procedure used for synthetic oligonucleotides.

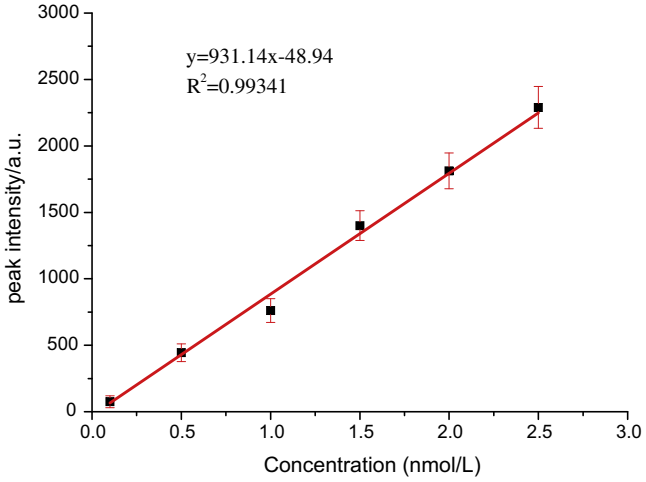

Fig. 5. Plot of DNA concentrations vs. detected signals.

PCR amplification

Asymmetric PCR, which predominantly produces Cy5.5-labeled ssDNA fragments, was utilized to amplify the target DNA for hybridization detection. The concentration ratio of reverse primer to forward primer was 5:1. Amplification was performed in a 20 μL reaction volume containing 10 μL of 2 × 1 Step Buffer (Takara Biotechnology (Dalian) Co., Ltd., China), 0.2 μL of 10 μM forward primer, 1 μL of 10 μM reverse primer, 3.8 μL of distilled water, and 5 μL of genomic template. PCR was performed using a Veritil 96-Well Thermal Cycler PCR system (Applied Biosystems) under the following conditions: 2 min at 94 °C; cycles of 20 s at 94 °C, 20 s at 55 °C, and 20 s at 72 °C; and a final extension of 5 min at 72 °C.

Real-time PCR amplification was performed using a LightCycler® 2.0 Real-Time PCR System (Roche), and a fluorescent PCR diagnosis kit for shigellosis (DAAN Gene Co., Ltd. of Sun Yat-sen University) was used in the assay.

Results and discussion

Specificity analysis of the sensor

To confirm the hybridization results and evaluate the fluorescence signals, 10 nM Cy5.5-labeled target DNA solutions, such as fully complementary DNA (FC TGT), one-base mismatched DNA (BPM TGT), non-complementary DNA (NC TGT), and fully complementary DNA containing no salt, were separately delivered on the fiber probe surface. Fig. 3 shows the typical fluorescence time trace during hybridization. Among the target DNA solutions, fully complementary DNA exhibited the highest fluorescence intensity. One-base mismatched DNA showed a small increase. Non-complementary DNA generated no obvious fluorescence signal, and no fluorescence signal was observed in the fully

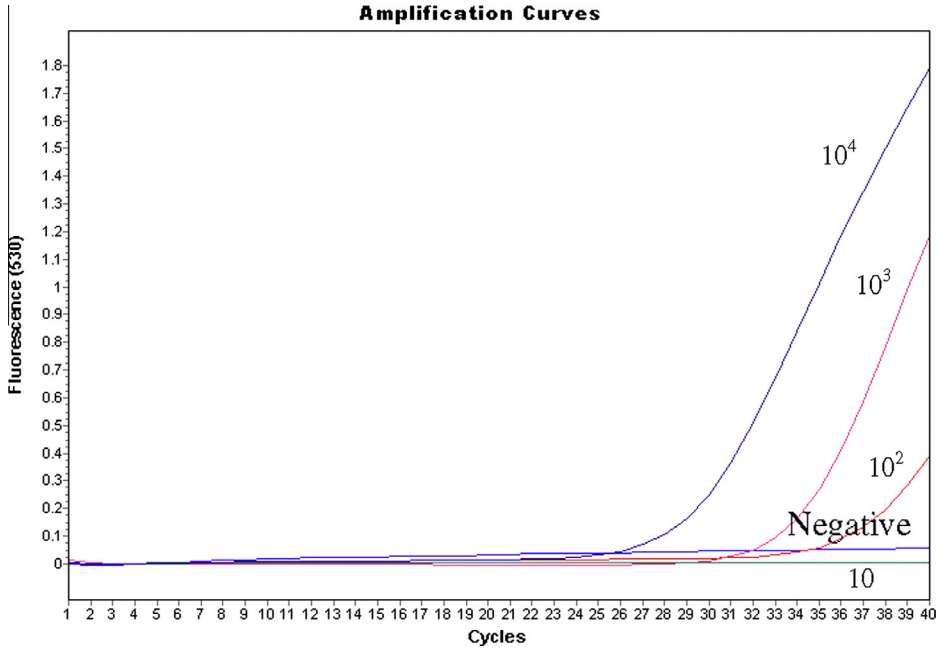

Fig. 6. Real-time PCR amplification.

complementary DNA solution containing no salt, suggesting that hybridization had not occurred. These results show that the observed fluorescence signal was generated from the specific DNA hybridization and not from non-specific adsorption or excitation of free Cy5.5-labeled DNA in the solution.

Detection sensitivity and regeneration of the sensor

High sensitivity is critical for developing a new biosensor with excellent performance. The regeneration ability of a surface-immobilized probe without significant loss of hybridization activity is also a desired feature of biosensors for practical applications. Thus, a series of concentrations of the synthesized complementary DNA was tested to investigate the detection sensitivity and regeneration performance of the portable evanescent wave fiber biosensor. Three consecutive regenerations were performed, and the obtained response profiles are plotted in Fig. 4. The detection limit for DNA hybridization was 10^{-10} M with a detection time of 5 min. In this study, the detection limit of biosensors is defined as three times the standard deviation of the mean blank values. In the range 0–2.5 nM, the fluorescent signal intensity increased linearly with DNA concentration, and the linear correlation coefficient was 0.9934, as shown in Fig. 5. For every detection concentration, the fiber probe surface was regenerated thrice with 0.5% SDS solution (pH 1.9). The regeneration time was 1 min. The immobilized recognition element was found to retain at least 30 successive assays, and the signal fluctuation was less than 5%.

Practical ability of the sensor

To verify the detection capability of the fiber optic biosensor, we compared the performance of real-time PCR with that of the fiber biosensor assay. The DNA from different concentrations of serially diluted *S. sonnei* (10^4, 10^3, 10^2, and 10 CFU/mL) was extracted. The result of real-time PCR amplification is shown in Fig. 6. The PCR blank control (deionized water as a DNA template) was also tested, and the sensitivity of real-time PCR was about 10^2 CFU/mL.

The asymmetric PCR products for *S. sonnei* (10^4, 10^3, 10^2, and 10 CFU/mL) were tested using the fiber biosensor. The time-dependent fluorescence signals resulting from the detection are shown in Fig. 7. Signal intensity was enhanced as the *Shigella* concentration increased.

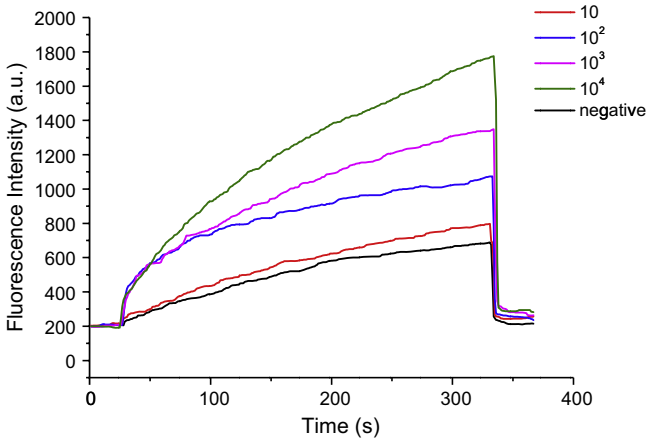

Fig. 7. Signal traces observed as the PCR products of different concentrations of templates.

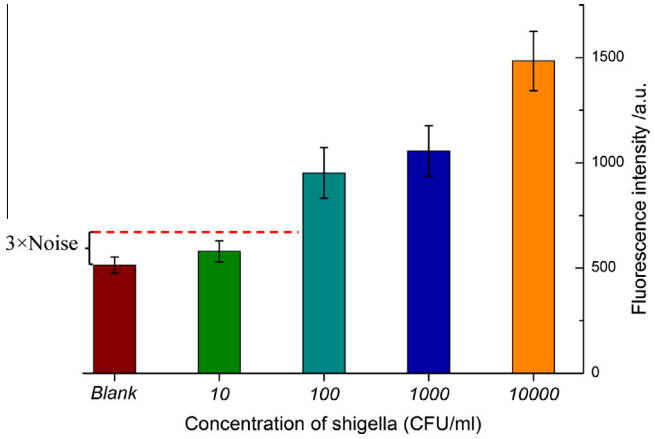

Fig. 8. Responses of the sensor to different PCR samples. The detection limit is 102 CFU/mL. Error bars indicate the standard deviation ($n = 3$).

The responses of the sensor to different PCR samples are shown in Fig. 8. The threshold for positive detection was set as the background (blank) signal + 3 × noise (standard deviation). Thus, the detection limit of fiber-optic biosensor was determined to be 10^2 CFU/mL, which shows that the sensitivity of the fiber-optic biosensor was similar to that of real-time PCR. However, the fiber biosensor is advantageous in terms of speed, simplicity, and suitability for on-site detection. In addition, the fiber biosensor can be applied for rapid detection of antibodies/antigens, heavy metal ions, toxins, and small-molecule analytes.

Conclusions

A reusable all-fiber evanescent wave biosensor system has been developed for the detection of *Shigella* with high sensitivity and specificity, rapidity, and simplicity. The detection of limit of this system is 0.1 nM (or 10^2 CFU/mL *Shigella*), which is similar to that of real-time PCR. However, our biosensor is advantageous in terms of speed, simplicity, and suitability for on-site detection. Moreover, the sensor surface can be reused more than 30 times without significant deterioration of performance, which is essential for the practical applications of biosensor.

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