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A fiber optic biosensor for specific identification of dead *Escherichia coli* O157:H7

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ABSTRACT

A compact and low-cost fiber optic biosensor was developed to specifically identify dead *Escherichia coli* (*E. coli*) O157:H7 with an antibody-activated combination tapered fiber as probe and propidium iodide (PI) as the fluorescent dye. The combined common multi-mode fiber devices and correlation detection significantly reduced the cost and increased the compactness of the instrument. The minimum detectable concentration of PI solution was lower than 1 ng mL^{-1}. Identification of dead bacteria was completed in 30 min, with 10 min allocated for the fiber probe to capture bacteria and 15 min for PI staining. The detection limit of dead *E. coli* O157:H7 was 10^4 cells mL^{-1} without interference from live *E. coli* O157:H7 and six other *E. coli* serotypes at 10^7 cfu mL^{-1} or cells mL^{-1}. Quantitation was achieved from 10^4 cells mL^{-1} to 10^7 cells mL^{-1} with r of 0.996 ($p < 0.0002$). This method integrating specific detection and dead cell identification could also be used to analyze other bacteria by coupling the biosensor with various antibodies and dyes.

1. Introduction

Significant progress in rapid and sensitive detection of bacteria has been observed in recent years [1–4]. However, differentiation of dead cells from live cells within a bacterial cell suspension continues to present a challenge despite its frequent application in cell proliferation assays [5], drug susceptibility testing [6], drug screening [7], among others.

Several methods have been developed to differentiate dead bacteria from live bacteria, with fluorescence-based staining as the most commonly used technique. Two fluorescent dyes have been used to perform differential staining of live and dead bacterial cells according to the integrity of their cell membranes. Fluorescence-based microscopes [8,9] were widely used in the past for signal analysis because they were easy to manipulate. However, the microscope usually provides qualitative results because count chamber-based quantitation is cumbersome, which limits its application. To address this restriction, fluorescence-based microplate readers [10], fluorometers [11], and flow cytometers [12] were developed for quantitative differentiation of dead and live bacteria. However, techniques using these instruments are either non-specific or dependent on highly complex equipment. New technologies have recently been developed, including polymerase chain reaction (PCR) coupled with ethidium bromide monoazide or propidium monoazide DNA dyes [13–15]. In this PCR-based method, the dyes penetrate the cell walls of dead or damaged cells and then bind to DNA to prevent amplification of the target sequence. This method exhibits high specificity and high sensitivity but is limited by cost and time requirement of detection. Alternatively, spectroscopy-based methods, such as Fourier transform-infrared spectroscopy [16], which provides the spectral fingerprints of microbial cells, use the variation in the composition and structure of dead and live bacteria to differentiate them. However, spectroscopy-based detection is difficult to use for direct on-site analysis of complex samples.

An alternative method uses fiber optic biosensors with an optical fiber as the light carrier and a fiber probe as the fluorescence detector. This approach has advantages such as compactness and miniaturization of the instrument, remote mode of detection in a

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hazardous environment, and resistance to electromagnetic interference [17], among others. Thus, fiber optic biosensors can be used in various fields such as medical science, biological engineering, food industry, and environmental monitoring [18–20]. A number of studies have recently reported on the use of fiber optic biosensors for detecting bacterial cells in suspension [21,22]. However, studies on the application of fiber optic biosensors to differentiate dead and live bacteria are rare. In the current study, a fluorescence-based fiber optic biosensor is developed for the specific identification of dead bacteria in suspension using propidium iodide (PI). PI is a membrane-impermeable dye that only penetrates membrane-damaged bacteria [23]. *Escherichia coli* (*E. coli*) O157:H7, which is a serious foodborne pathogen and can be transmitted among people via the fecal–oral route leading to hemorrhagic diarrhea and even kidney failure, was used to evaluate the sensitivity and specificity of the proposed method and set the basis for its future on-site surveillance. The proportion of dead bacteria with the total bacteria can be differentiated. This technique can also be used to differentiate dead cells from live cells (or vice versa) of other bacteria by coupling the biosensors with other fluorescent dyes and specific antibodies.

2. Materials and methods

2.1. Chemicals and reagents

(3-Aminopropyl)triethoxysilane (APTES), glutaraldehyde, bovine serum albumin (BSA), and PI were purchased from Sigma–Aldrich (Gillingham, Dorset, UK). Unless otherwise specified, all other reagents were supplied by the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), of analytical grade, and were used without further purification. A PI stock solution with concentration of 1 mg mL^{-1} was prepared in distilled deionized water and stored at 4 °C. PI working solutions with varying concentrations were diluted from the PI stock solution using 0.01 mol L^{-1} phosphate-buffered saline (PBS, pH 7.2).

2.2. Bacterial culture and monoclonal antibody preparation

E. coli O6:H6, *E. coli* O44:K74, *E. coli* O78:H11, *E. coli* O114:K90, *E. coli* O128:K67, *E. coli* O144, and *E. coli* O157:H7 were previously preserved in our laboratory. Prior to the study, these bacterial strains were cultured in Luria-Bertani (LB) broth at 37 °C until the exponential growth phase and then collected by centrifugation at 6000 rpm (Allegra X-22R, Beckman, Germany) for 5 min. The pellet was resuspended in sterile saline (0.85% salt solution) to a final concentration of ~10^8 colony forming units (cfu, which is an estimate of viable bacterial numbers usually existing in the form of colony) mL^{-1} for use, as determined by plate count. Bacterial suspensions with serial concentrations were also prepared by serial saline dilution.

Balb/c mice were intraperitoneally immunized with 10^6 inactivated cells of *E. coli* O157:H7 thrice at two-week intervals. After evaluating the immune response, the splenocytes from immunized mice were fused with logarithmically growing mouse myeloma cells Sp2/0 according to polyethylene glycol method [24]. The culture supernatants were screened by indirect ELISA, with *E. coli* O157:H7 as specific coated antigen and other serotypes of *E. coli* as the specificity control, and were cloned by limited dilution method [25]. Balb/c mice were intraperitoneally immunized with hybridoma cells, and the ascites was collected. Purification was performed by acid-ammonium sulfate method, and the purified monoclonal antibodies (Reference No. 7D9) with ELISA titer no less than 1:128,000 were stored at −20 °C.

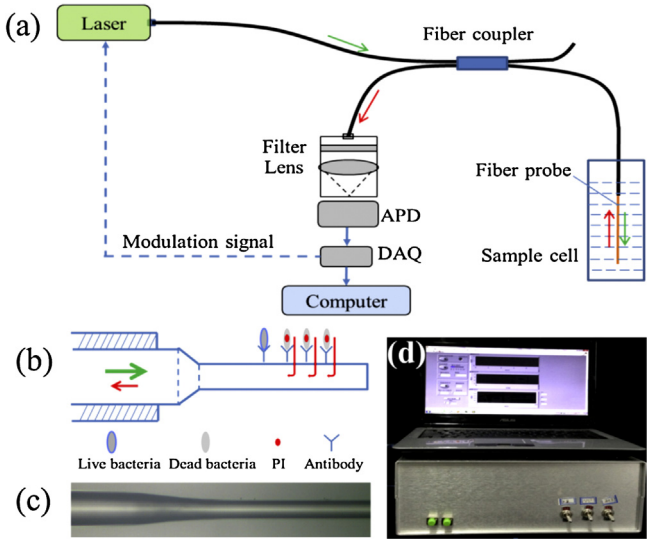

Fig. 1. Principle of the fiber optic biosensor. (a) Schematic representing the fiber optic biosensor. The excitation light with wavelength of 532 nm generated by a solid laser was transmitted into the fiber probe through the fiber coupler. The fluorescence was then collected and propagated back through fiber coupler, filter lens, APD, and DAQ system to the computer. (b) Identification of dead bacteria using the fiber probe. The dead and live bacteria were captured by the antibodies immobilized on the surface of the probe. PI penetrated the cell membrane of dead bacteria and produced detectable fluorescence with excitation by 532 nm light. (c) Photograph of the combination tapered fiber probe. The lengths of the tapered section and the sensing region were approximately 0.03 cm and 2.5 cm, respectively. (d) Prototype of the fiber optic biosensor. All the components (a) were encapsulated in the box, except the fiber probe and sample cell. The fiber probe had a FC connector which could be connected to the fiber coupler by the fiber adapter in the front-panel of the box.

2.3. Design of the instrument

To avoid problems regarding free space beams and optical alignment, common multimode quartz optical fibers (NA = 0.22) with a core diameter of 105 μm and a cladding diameter of 125 μm were used for light transmission in the configuration of biosensor. Then, optical fibers were connected to one another by fiber adapters. To enhance signal resolution, a correlation detection based on phase-locked filtering algorithm, which was performed with LabVIEW, was introduced into the system.

Given that the input signal $x(t)$ consists of the desired signal $s(t)$ and noise $n(t)$, the cross-correlation function of $x(t)$ and the reference signal $r(t)$ are expressed as follows:

$$R_{xr}(\tau) = \lim_{T\to\infty}\frac{1}{T}\int_{-T/2}^{T/2} x(t)r(t-\tau)dt$$

$$= \lim_{T\to\infty}\frac{1}{T}\int_{-T/2}^{T/2} (s(t)+n(t))r(t-\tau)dt = R_{sr}(\tau)+R_{nr}(\tau) \quad (1)$$

The correlation function $R_{nr}(\tau)$ of noise $n(t)$ would be zero as there was no correlation between the noise and the reference signal. Thus, the final signal $R_{xr}(\tau)$ only contained the correlation function $R_{sr}(\tau)$ of signal $s(t)$ so that the desired signal could be extracted. This approach enhanced the sensitivity to simplify data processing and reduced the number of discrete components to enhance the integrity of the system.

The schematic of the fiber optic biosensor is shown in Fig. 1(a). A 532 nm solid laser (Shanghai Fiblaser Technology Co., Ltd., Shanghai, China) with a modulation frequency of 1 kHz was used as the excitation light source because the peak excitation wavelength of PI is at 500 nm. The pigtail fiber of the light source was directly launched into a fiber coupler (2 × 2, couple ratio: 20/80), and the