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Borosilicate Glass Fiber-Optic Biosensor for the Detection of *Escherichia coli*

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Abstract Polyclonal antibodies against *Escherichia coli* and fluorescent, secondary, antibodies were immobilized on borosilicate glass fibers pre-treated with 3-glycidyloxypropyl trimethoxysilane (GPS). Light with an average wavelength of 627 nm, emitted by a diode placed at one end of the glass fiber, was detected by an ultrasensitive photodiode with peak sensitivity at 640 nm. Changes in fluorescence, caused by binding of *E. coli* to the antibodies, changed the net refractive index of the glass fiber and thus the internal reflection of light. These evanescent changes in photon energy were recorded by an ultrasensitive photodiode. Signals were amplified and changes in voltage recorded with a digital multimeter. A linear increase in voltage readings was recorded over 2 h when 3.0×10^7 CFU/ml and 2.77×10^9 CFU/ml *E. coli* were adhered to the antibodies. Voltage readings were recorded with *E. coli* cell numbers from 2×10^3 CFU/ml to 2×10^6 CFU/ml, but readings remained unchanged for 2 h, indicating that the limit of detection is 3.0×10^7 CFU/ml. This simple technology may be used to develop a low-cost, portable, fiber-optic biosensor to detect *E. coli* in infections and may have applications in the medical field. Research is in progress to optimize the sensitivity of the fiber-optic biosensor and determine its specificity.

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Introduction

Escherichia coli is one of the most frequent causes of cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), traveler's diarrhea, neonatal meningitis, and pneumonia [24]. Enteropathogenic *E. coli* (EPEC) is associated with childhood diarrhea and enterotoxigenic *E. coli* (ETEC) is responsible for 11–15% of traveler's diarrhea [24]. Some reports list the latter to be as high as 30–45% [13]. The development of a rapid, low-cost, and preferably portable, biosensor to detect changes in *E. coli* cell numbers is of the essence.

Biosensors are defined as "self-contained, integrated devices capable of providing specific quantitative or semi-quantitative analytical information" in the presence of biological analytes [7, 21]. The mode of bio-recognition and type of transducer used in biosensors defines its practical application [6]. Typical examples of bio-recognition elements include proteins, enzymes, antibodies, aptamers, membrane pores and channels, ionophores, and receptors [3, 8, 22, 23].

In labeled biosensors, analytes are detected by using reporter molecules such as antibodies and fluorescent markers [12]. Apart from being time-consuming, readings recorded with labeled biosensors are often non-specific, mostly due to background signals generated by reporter molecules [12]. Optical transducers, on the other hand, make use of sensors that detect absorbance [9], reflectance, fluorescence [2, 10], chemiluminescence, evanescent wave, or bioluminescence [2, 15]. Other more sophisticated sensors make use of fiber optics, lateral flow assays (LFAs),

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surface plasmon resonance (SPR), and enzyme-linked immunosorbent assays (ELISAs) [18].

Fiber-optic biosensors use light to interact with target antigens. In the fiber-optic biosensor developed by Bharadwaj et al. [1], light enters the fiber and is transported by total internal reflectance (TIR). An evanescent wave (D_P), which penetrates the core of the fiber, interacts with an antibody immobilized on the surface. Binding of more bacterial cells to the surface of the fiber leads to a change in light intensity, which is recorded by a spectrometer [1]. In this design, the fiber was U-bent to increase the penetration depth of the evanescent wave [1].

A fiber-optic biosensor using tapered optical fibers immobilized with antibodies was developed to detect *E. coli* with a limit of detection (LOD) of 70 CFU/ml [19]. The biosensor was re-useable, as bacterial cells were released from the surface by changing the pH [19]. Kuswandi and co-workers [9] used a combination of light, optical fiber, and a spectrophotometer to detect analytes [9]. DeMarco and Lim [5] developed an optical fiber-based biosensor to detect evanescent waves generated by cells of *E. coli* in ground beef. Ohk and Bhunia [17] used a multiplex of fiber-optic cables to develop a biosensor that detected a number of different microorganisms, including *E. coli* O157:H7. This was done by immobilizing different antibodies on separate optical fibers and recording the fluorescence intensity for each of the fibers [17].

The biggest challenge in developing a biosensor is to design a transducer sensitive enough to detect small changes in biologically active molecules or microbial cell numbers [14, 20]. Mura et al. [16] developed a biosensor from mesoporous thin-film of titanium, treated with (3-amino-propyl)triethoxysilane (APTES) and glutaraldehyde (GA), and coated with antibodies against *E. coli.* Although *E. coli* cell numbers as low as 100 CFU/ml were detected, the sensor was not portable as readings are recorded using FTIR (Fourier transform infrared) spectroscopy. Ohk and Bhunia [17] developed a multiplex fiber optic biosensor to detect bacterial cells, including *E. coli* O157:H7, in meat samples. Antibodies were immobilized onto optical fibers and changes in cell numbers were recorded by reading variations in fluorescence intensities. The limit of detection was 10^3 CFU/ml for *E. coli* O157:H7 and *Salmonella enterica* [17].

In this study, we describe the construction of a simple borosilicate glass fiber-optic biosensor to identify *E. coli* and detect changes in cell numbers. Hydroxylated borosilicate glass fibers were treated with GPS to form a self-assembled monolayer. Primary antibodies against *E. coli* were covalently bound to the GPS monolayer and linked to fluorescent secondary antibodies. Changes in the intensity of fluorescence when cells of *E. coli* attached to the immobilized antibodies were detected by an ultrasensitive photodiode. The signals were amplified and voltage readings were recorded by using a digital multimeter.

Materials and Methods

Preparation of Borosilicate Fibers

Optical fibers were manufactured from borosilicate glass (84% SiO_2 and 11% B_2O_3). One end of a borosilicate glass rod was heated in a butane flame and tapped with a metal rod to extrude thin glass fibers. The fibers were allowed to cool, cut to the desired length, hydroxylated by immersion into sulfuric acid and hydrogen peroxide (3:1 ratio) for 10 s, and then immersed in toluene (99.8%, v/v). The fibers were rinsed twice in phosphate-buffered saline (PBS, pH 7.4) and dried under a flow of N_2.

Immobilization of Antibodies onto Glass Fibers

Primary antibodies were immobilized onto the surface of the borosilicate glass fibers according to the method described by Corso and co-workers [4]. In short, the hydroxylated borosilicate glass fibers were exposed to 4% (v/v) GPS (dissolved in 99.8%, v/v, toluene), flushed with N_2, sealed, and left at 25° C for 22 h to form a self-assembled monolayer (SAM) of GPS on the surface (a schematic representation of the glass fiber surface treatment is shown in Fig. 1). The fibers were then rinsed twice with sterile PBS (pH 7.4) to remove unbound GPS and immersed for 4 h at 25 °C in 750 ml PBS (pH 7.4) that contained 200 µg/ml primary polyclonal *E. coli* antibodies (PAb, Abcam, Cambridge, United Kingdom). Binding of antibodies to the GPS layer was conducted in an opaque container, flushed with N_2. The fibers were then rinsed with PBS (pH 7.4) to remove unbound antibodies and exposed to secondary, fluorescent, antibodies (2 mg/ml donkey polyclonal Secondary Antibody to Goat IgG—H and L; Alexa Fluor 488, EX at 495 nm and EM at 519 nm obtained from Abcam). Incubation was at 4 °C for 2 h in a light-tight container. The fibers were rinsed with large volumes of sterile PBS (pH 7.4) to remove unbound secondary antibodies.

Preparation of *E. coli* Cells and Binding to Immobilized Antibodies on Glass Fibers

Escherichia coli DH5α, obtained from the culture collection of the Department of Microbiology, Stellenbosch University, was grown in Nutrient Broth (Biolab, Biolab Diagnostics, Midrand, SA) at 37 °C for 24 h. The cells were harvested (8000×*g*, 2 min), rinsed twice with sterile

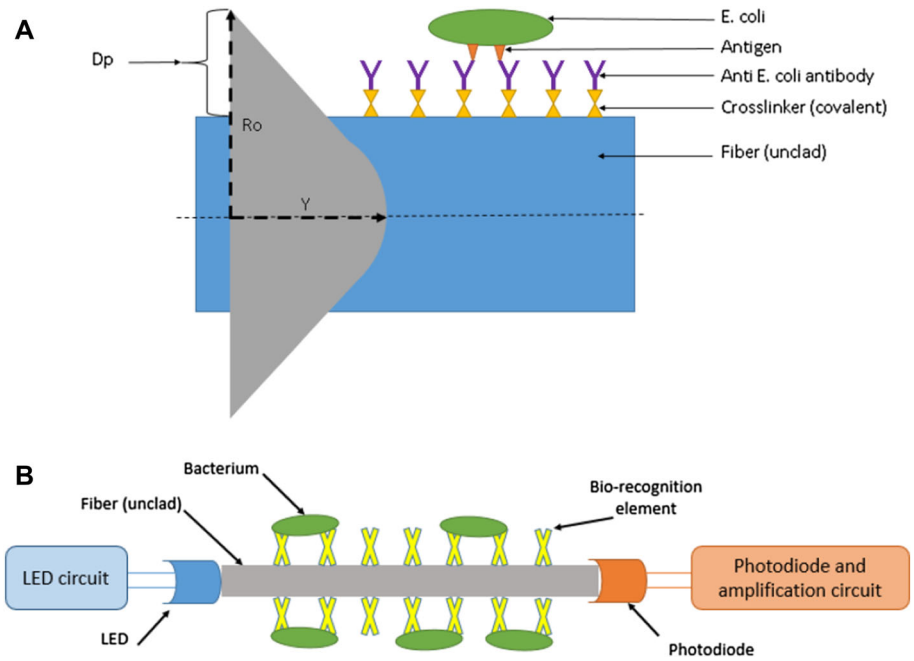

Fig. 1 Schematic representation of the glass fiber-optic biosensor with antibodies immobilized on the surface. As light travels through the glass fiber, the evanescent field interacts with the bacteria on the surface of the fiber. **a** A cross-section representation of the fiber and **b** a representation of the initial bacterial test setup

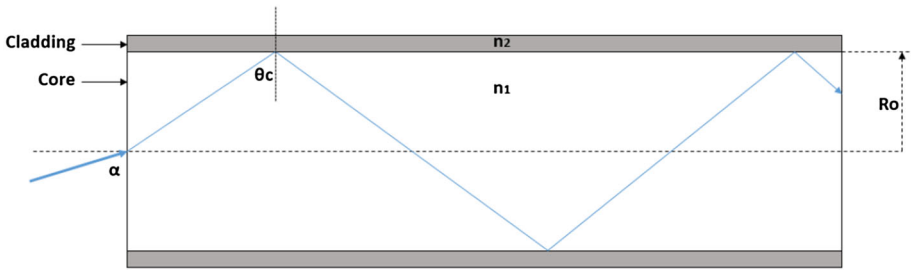

Fig. 2 Ray Tracing model of light traveling through an optical fiber. The two respective refractive indices are indicated as n_1 and n_2. R_O is the fiber core radius, α is the angle of incidence of the fiber, and θ_c is the critical angle of light exiting the fiber

PBS (pH 7.4), and re-suspended in PBS. Cells were serially diluted in sterile PBS (pH 7.4), plated onto Nutrient Agar (Biolab), and colonies counted after 24 h of incubation at 37 °C.

To determine binding of *E. coli* to primary antibodies, the GPS-treated borosilicate glass fibers were exposed to 500 µl of washed cells (1×10^8 CFU/ml) and were allowed to adhere to the immobilized antibodies at 25 °C for 1, 4, and 5 h, respectively. The fibers were then washed with 1 ml sterile PBS (pH 7.4) to remove unbound cells. Cells attached to the fluorescent secondary antibodies were visualized by using a wide-field fluorescence microscope (Olympus IX81 with UBG excitation filter exited at 492 nm). Areas of fluorescence were randomly selected and cropped to the same size. Background readings were subtracted and fluorescent readings calculated by taking into consideration readings recorded with untreated glass fibers. The state of viability of cells attached to the glass fibers was recorded by staining the cells with propidium iodide and Syto9, using the BacLight LIVE/DEAD Bacterial viability kit (Invitrogen, Carlsbad, USA). Propidium iodide stained non-viable cells red and Syto9 viable cells green. Visualization was by fluorescent microscopy, with excitation wavelengths from 492 to 572 nm. Images were recorded over a period of 2–5 min.

Biosensor Setup

A schematic representation of the glass fiber-optic biosensor, with antibodies immobilized on the surface, is shown in Fig. 1a. As light emitted from the LED circuit travels through the glass fiber (Fig. 1b), the evanescent field interacts with the bacteria on the surface of the fiber. The light refraction, calculated from refractive indices explained by the Ray Tracing model [11] and schematically represented in Fig. 2, is recorded by a photodiode.

The numerical aperture of an optical fiber is described by

$$\mathrm{NA} = \sqrt{n_1^2 - n_2^2}, \tag{1}$$

where n_1 and n_2 are the two respective refractive indices. The critical angle of light entering and exiting the fiber is described by

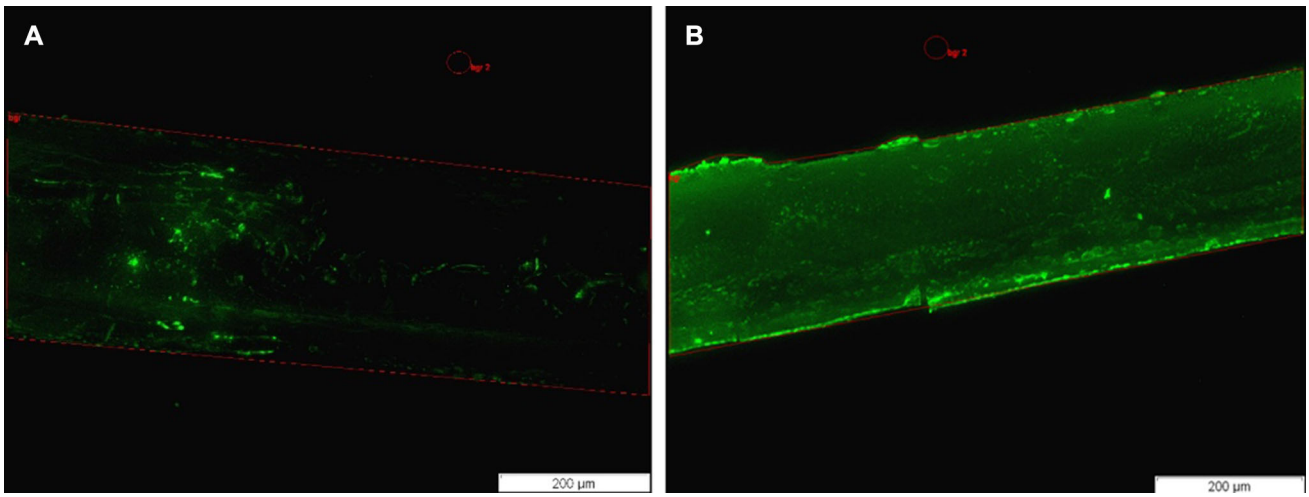

Fig. 3 **a** SEM image (×10 magnification) showing the distribution of secondary antibodies on a glass fiber coated with GPS only (no primary antibodies) and **b** Z-stack fluorescence microscopy (×10 magnification) showing the distribution of *E. coli* cells adhered to primary and secondary antibodies on a glass fiber pre-coated with GPS

$$\theta_c = \sin^{-1}\left(\frac{n_1}{n_2}\right). \quad (2)$$

The angle of incidence of the fiber, α, must satisfy the equation

$$\alpha > \theta_c, \quad (3)$$

for total internal reflection (TIR) to occur in the fiber.

The Ray Tracing model describes the penetration depth (D_p) as the length of the evanescent field exiting the physical fiber core. This is described as

$$D_p = \frac{\lambda}{2\pi\sqrt{n_1^2 \sin^2(\alpha) - n_2^2}}, \quad (4)$$

where λ is the wavelength of light, n_1 is the refractive index of the fiber, n_2 is the refractive index of the surrounding medium, and α is the angle of incidence of light.

The LED, shown in Fig. 1, had a peak wavelength of 627 nm and the photodiode (PD) in Fig. 1 a peak sensitivity of 640 nm. The two adapters that housed the LED and PD were 3-D printed. The LED was connected to an LED circuit consisting of a power source, a resistor, and a LED. The photodiode output current was converted to voltage and amplified. This was connected to an Agilent 6.5 digit Digital Multimeter. The setup was placed in a darkroom and covered with a light shield. Cells of *E. coli*, suspended in 250 µl PBS to contain 2.77×10^9 CFU/ml, 3.0×10^7 CFU/ml, 2.0×10^6 CFU/ml, 2.0×10^5 CFU/ml, 2.0×10^4 CFU/ml, and 2.0×10^3 CFU/ml, respectively, were pipetted onto the fibers suspended above the glass slides. Voltage measurements were taken at specific time points for 2 h.

Results and Discussion

The mean diameter of the hydroxylated borosilicate glass fibers was 243 µm, with a standard deviation of 45 µm. No fluorescence was detected when the fibers were treated with secondary antibodies only (no GPS and no primary antibodies) and when treated with GPS only, i.e., no primary and secondary antibodies (not shown). The irregular distribution of secondary antibodies on the surface of a GPS-coated glass fiber, in the absence of primary antibodies, is shown with Z-stack fluorescent microscopy (Fig. 3a). Structural variations in glass fiber surfaces may be ascribed to the irregular binding of GPS to hydroxyl groups. This may lead to clumping of the primary and secondary antibodies to GPS. Surface irregularities may also form during the extrusion of the glass fibers, or the deposition of impurities onto the finished surfaces.

Strong binding of *E. coli* to antibody-coated GPS and the equal distribution of *E. coli* cells along the fibers, observed with Z-stack fluorescent microscopy (Fig. 3b), suggests that secondary antibodies were successfully immobilized to primary antibodies. Most of the cells remained viable for at least 5 h (stained green) when attached to secondary antibodies, as shown by large numbers of green fluorescent cells clumped to the antibodies (Fig. 4c). Viable and actively dividing cells produce more antigens and should adhere stronger to fibers than metabolically inactive cells. Furthermore, metabolically active cells of *E. coli* are more mobile than stationary-phase cells and will compete more successfully for adherence to antibodies.

A linear increase in voltage readings was recorded with prolonged exposure of *E. coli* cells to antibodies

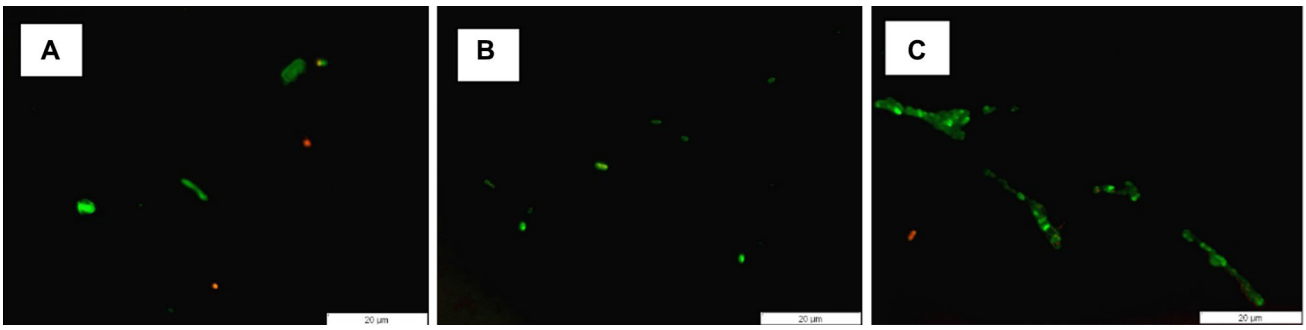

Fig. 4 Fluorescent microscopy images of *E. coli* attached to glass fibers treated with GPS, primary antibodies, and secondary antibodies for **a** 1 h, **b** 4 h, and **c** 5 h. Cells were stained using the BacLight LIVE/DEAD Bacterial viability kit (Invitrogen). Propidium iodide stained non-viable cells red and Syto9 viable cells green (Color figure online)

immobilized on the borosilicate glass fibers (Fig. 5a, b). The initial cell concentration had little effect on the rate at which the voltage readings increased, as observed with initial cell numbers of 2.77×10^9 CFU/ml (Fig. 5a) and 3.0×10^7 CFU (Fig. 5b). The readings were reproducible, with less than 10% variation. The number of cells binding to the antibodies, whether due to prolonged incubation or due to an increase in cell numbers, is directly related to an

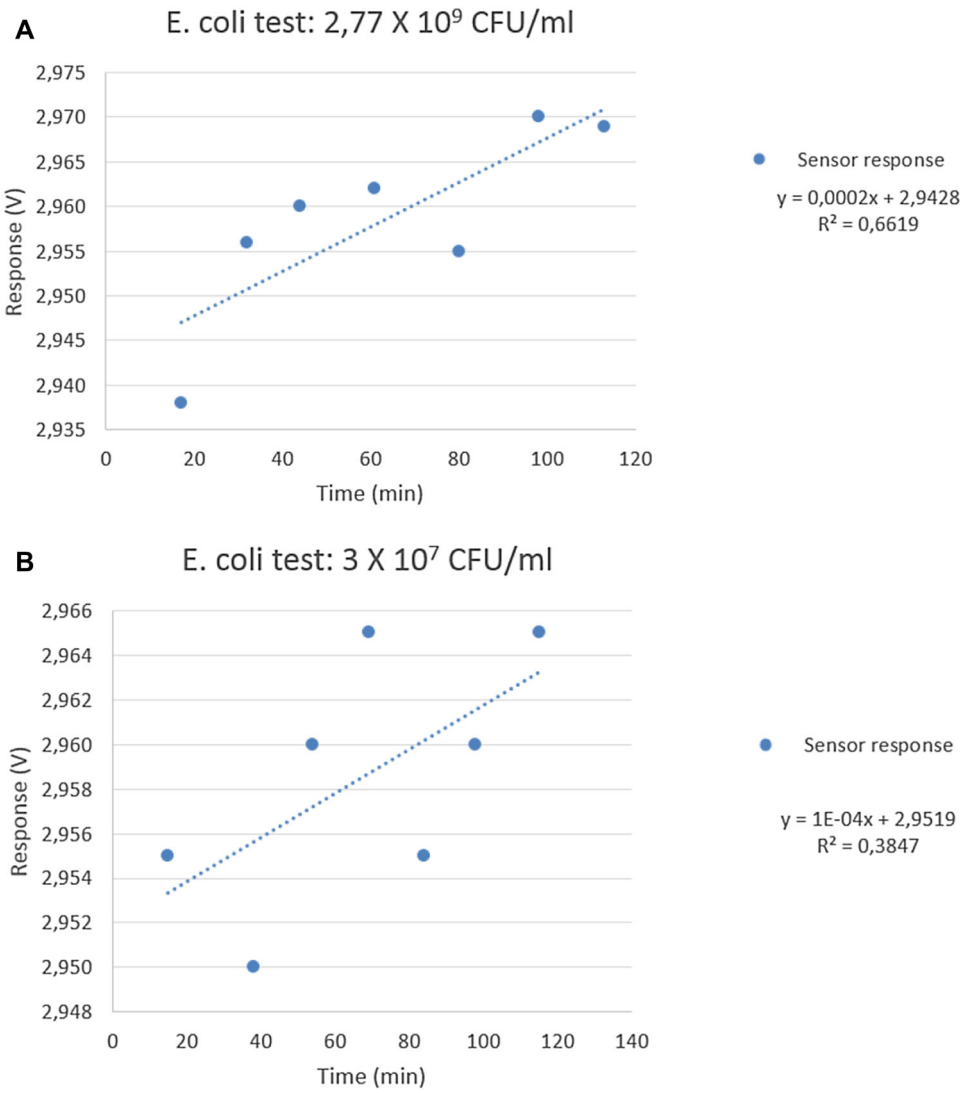

Fig. 5 Voltage readings recorded with **a** 2.77×10^9 CFU/ml and **b** 3×10^7 CFU/ml *E. coli*. Readings were reproducible, with less than 10% variation

increase in voltage. Based on these results, it is safe to assume that the number of antibody binding sites superseded the number of bacterial cells. Voltage readings were recorded with lower cell numbers of *E. coli* (from 2×10^3 CFU/ml to 2×10^6 CFU/ml), but readings remained unchanged for 2 h. This indicated that the limit number of cells that could be detected with the borosilicate glass fiber biosensor was 3.0×10^7 CFU/ml.

Although the borosilicate glass fiber biosensor we have developed is not as sensitive as the fiber optic biosensor described by Ohk and Bhunia [17] with a detection limit of 10^3 CFU/ml *E. coli*, the technology used to design the biosensor is simple and allows for the developing of a low-cost and portable detection device. The sensor we developed may be used to detect *E. coli* in infections and may have applications in the medical field. Research is in progress to optimize the sensitivity of the fiber-optic biosensor and to determine its specificity.

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References

1. Bharadwaj R, Sai V, Dhawangale KTA, Kundu T, Titus S, Verma PK, Mukherji S (2011) Evanescent wave absorbance based fiber optic biosensor for label-free detection of *E. coli* at 280 nm wavelength. Biosens Bioelectron 26(3367–3370):1
2. Bosch ME, Sánchez AJR, Rojas FS, Ojeda CB (2007) Recent development in optical fiber biosensors. Sensors 7(797–859):2
3. Chambers JP, Arulanandam BP, Matta LL, Weis A, Valdes JJ (2008) Biosensor recognition elements. Curr Issues Mol Biol 10(1–12):3
4. Corso C, Dickherber A, Hunt W (2008) An investigations of antibody immobilization methods employing organosilanes on planar ZnO surfaces for biosensor applications. Biosens Bioelectron 24(805–811):4
5. DeMarco D, Lim D (2002) Detection of *Escherichia coli* O157:H7 in 10- and 25-gram ground beef samples with an evanescent-wave biosensor with silica and polystyrene waveguides. J Food Prot 65(596–602):5
6. Hunt HK, Armani AM (2010) Label-free biological and chemical sensors. Nanoscale 2(1544–1559):6
7. Ivnitski D, Abdel-Hamid I, Atanasov P, Wilkins E (1999) Biosensors for detection of pathogenic bacteria. Biosens Bioelectron 14(599–624):7
8. Kim P, Kohli N, Hassler B, Dotson N, Mason A, Worden RM, Ofoli R (2003) An electrochemical interface for integrated biosensors. Proc IEEE 2(1036–1040):8
9. Kuswandi B, Nuriman, Huskens J, Verboom W (2007) Optical sensing systems for microfluidic devices: a review. Anal Chim Acta 601(141–155):9
10. Lazcka O, Del Campo FJ, Muñoz FX (2007) Pathogen detection: a perspective of traditional methods and biosensors. Biosens Bioelectron 22(1205–1217):10
11. Leung A, Shankar PM, Mutharasan R (2007) A review of fiber-optic biosensors. Sens Actuators, B 125(688–703):11
12. Luo X, Davis JJ (2013) Electrical biosensors and the label free detection of protein disease biomarkers. Chem Soc Rev 42(5944–5962):12
13. Madappa T (2017) *Escherichia coli* infections, Medscape, http://emedicine.medscape.com/article/217485-overview. Accessed on July 2017)
14. McGraw SK, Anderson MJ, Alocilja EC, Marek PJ, Senecal KJ, Senecal AG (2011) Antibody immobilization on conductive polymer coated nonwoven fibers for biosensors. Sens Transducers 13(142–149):9n
15. Monk DJ, Walt DR (2004) Optical fiber-based biosensors. Anal Bioanal Chem 379(931–945):13
16. Mura S, Greppi G, Marongiu ML, Roggero PP, Ravindranath SP, Mauer LJ, Schibeci N, Perria F, Piccinini M, Innocenzi P, Irudayaraj J (2012) FTIR nanobiosensors for *Escherichia coli* detection. Beilstein J Nanotechnol 3:485–492
17. Ohk S-H, Bhunia AK (2013) Multiplex fiber optic biosensor for detection of *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella enterica* from ready-to-eat meat samples. Food Microbiol 33(166–171):14
18. Queiros RB, Noronha J, Marques P, Sales MGF (2012) Emerging (bio)sensing technology for assessing and monitoring freshwater contamination—methods and applications. In: Voudouris D (ed) Ecological water quality—water treatment and reuse, vol 15. InTech, Rijeka, pp 65–94
19. Rijal K, Leung A, Shankar PM, Mutharasan R (2005) Detection of pathogen *Escherichia coli* O157:H7 at 70 cells/mL using antibody-immobilized biconical tapered fiber sensors. Biosens Bioelectron 21(871–880):16
20. Strehlitz B, Nikolaus N, Stoltenburg R (2008) Protein detection with aptamer biosensors. Sensors 8(4296–4307):10n
21. Thévenot DR, Toth K, Durst RA, Wilson GS (1999) Electrochemical biosensors: recommended definition and classification. International Union of Pure and Applied Chemistry 17
22. Trojanowicz M (2001) Miniaturized biochemical sensing devices based on planar bilayer lipid membranes. Fresenius J Anal Chem 371(246–260):18
23. Vo-Dinh T, Cullum B (2000) Biosensors and biochips: advances in biological and medical diagnostics. Fresenius J Anal Chem 366(540–551):19
24. Werber D, Krause G, Frank C, Fruth A, Flieger A, Mielke M, Schaade L, Stark K (2012) Outbreaks of virulent diarrheagenic *Escherichia coli*—are we in control? BMC Medicine 10:11. http://www.biomedcentral.com/1741-7015/10/11