Detection of water-borne E. coli O157 using the integrating waveguide biosensor

Peixuan Zhu a, *, Daniel R. Shelton b, Jeffrey S. Karns b, Appavu Sundaram b, Shuhong Li a, Pete Amstutz a, Cha-Mei Tang a

a Creatv MicroTech, Inc., 11609 Lake Potomac Drive, Potomac, MD 20854, USA
b Environmental Microbial Safety Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705, USA

Received 16 October 2004; received in revised form 11 December 2004; accepted 4 January 2005
Available online 16 February 2005

Abstract

Escherichia coli O157:H7, the most common serotype of enterohemorrhagic E. coli (EHEC), is responsible for numerous food-borne and water-borne infections worldwide. An integrating waveguide biosensor is described for the detection of water-borne E. coli O157, based on a fluorescent sandwich immunoassay performed inside a glass capillary waveguide. The genomic DNA of captured E. coli O157 cells was extracted and quantitative real-time PCR subsequently performed to assess biosensor-capture efficiency. In vitro microbial growth in capillary waveguide is also documented. The biosensor allows for quantitative detection of as few as 10 cells per capillary (0.075 ml volume) and can be used in conjunction with cell amplification, PCR and microarray technologies to positively identify a pathogen.

Keywords: Integrating waveguide biosensor; E. coli O157:H7; Sandwich immunoassay; Real-time PCR

1. Introduction

Enterohemorrhagic Escherichia coli (e.g., E. coli O157:H7) is a major food-borne and water-borne pathogen that causes diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (Su and Brandt, 1995). Outbreaks have occurred in many developed countries, including Canada, Europe, Australia, and Japan. The Center for Disease Control and Prevention (CDC) estimates that E. coli O157:H7 causes nearly 75,000 human infections in the U.S. each year (Mead et al., 1999).

Many methods have been developed to detect E. coli O157:H7 in food and water matrices, including traditional culturing with selective media (Hammack et al., 1997; March and Ratnam, 1986), serotyping with specific antibodies to O157 and H7 antigens (Chapman et al., 1997; Czajka and Batt, 1996; Shelton and Karns, 2001; Tomoyasu, 1998), amplification of specific genes by PCR (Higgins et al., 2003; Johnson and Stell, 2001; Maurer et al., 1999; Wang et al., 2002) or hybridization of virulence genes by DNA microarrays (Bekal et al., 2003). Each method has limitations with respect to sensitivity, specificity, and quantitation. Consequently, multiple assays are required to detect and quantify small numbers of water-borne E. coli O157:H7 and confirm strain identity.

The integrating waveguide biosensor was originally developed by Ligler et al. (2002) at the Naval Research Laboratory, Washington, DC. The biosensor utilizes a sandwich antibody technique for capture and detection, with the capture antibodies attached to the inner surface of a glass capillary tube. Detection and quantitation are achieved by illuminating the capillary tube (i.e., optical waveguide) at a 90° angle relative to the length of the waveguide and subsequent collection
Fig. 1. Side view of waveguide capillary and experimental configuration of the integrating waveguide biosensor. A sandwich immunoassay is performed on the inner surface of capillary using Cy5-labelled anti-\(E. coli\) O157 antibody for generation of the specific fluorescent signal. Lines with letters 1.66, 75 and 50 mm indicate the diameter, length and laser excitation area of the capillary, respectively.

of the emitted fluorescence from the end of the waveguide (Fig. 1). Initial results gave a detection limit of 40 pg ml\(^{-1}\) for mouse IgG and 30 pg ml\(^{-1}\) for staphylococcal enterotoxin B (SEB) in the sandwich assays (Ligler et al., 2002), which is more sensitive than other fiber optic and array biosensors (Anderson et al., 1994; Rowe et al., 1999).

Compared to the existing technologies, the integrating waveguide biosensor provides a platform with multiple advantages for the detection of \(E. coli\) O157:H7 and other analytes in low concentration. Although the capillary tube was originally envisioned only as a waveguide, it is readily available, convenient for laboratory experimentation, and compatible with other sample preparation and detection protocols.

The capillary tube can serve as an incubation vessel for growth of bacterial pathogens after capture, allowing for confirmation of viability, as well as amplification and retrieval for further characterization. The enclosed structure of capillary tubes is of particular benefit when dealing with pathogenic substances. In addition, clean up of the contaminants by washing, followed with in vitro lysis of pathogens, allows for rapid confirmation of strain identity using PCR or microarray technologies.

2. Materials and methods

2.1. Chemicals and reagents

NeutrAvidin™ was purchased from Pierce Biotechnology (Rockford, IL). One milligram of monoclonal anti-\(E. coli\) O157 antibody solution (BioDesign, Saco, Maine) was conjugated with Sulfo-NHS-LC-Biotin (Pierce Biotechnology, Rockford, IL) according to the manufacturer’s instructions. One milligram of goat anti-\(E. coli\) O157:H7 antibody (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was dissolved in 1 ml of phosphate-buffered saline (PBS) was conjugated with Cy5 dye using the FluoroLink-Ab Cy5 labeling kit (Amersham Biosciences, Piscataway, NJ) according to the manufacturer’s instructions.

2.2. Bacterial strain and growth conditions

\(E. coli\) O157 strain 2B, isolated from the Gwynns Falls watershed (Baltimore County, MD), was used for all experiments. This strain has been deposited with the \(E. coli\) Reference Center, Pennsylvania State University (# 2.4166). The culture was routinely grown at 37°C in minimal lactose broth medium with yeast extract (MLB-Y) for 16 h (Shelton et al., 2003). At stationary phase the cell concentration was ca. 5 \(\times\) 10\(^8\) cells ml\(^{-1}\) as determined using a hemocytometer (Hausser Scientific, Gaithersburg, MD). Growth curves in capillary tubes and in 15 \(\times\) 125 mm test tubes (Kimble/Kontes, Vineland, NJ) containing 10 ml of broth were conducted at 44°C. Viable cell counts were determined by plating on MacConkey agar using an Autoplate® 4000 (Spiral Biotech, Norwood, MA). After overnight incubation at 37°C, colonies were counted using a QCount (Spiral Biotech).

2.3. Biosensor sandwich immunoassays

Glass capillary tubes (75 mm long, 1.66 mm O.D., 1.23 mm I.D.) were obtained from Drummond Scientific Company (Broomall, PA). The tubes were prepared according to the procedure described (Ligler et al., 2002). Briefly, capillary tubes were cleaned with methanol/HC1 and sulfuric acid, dried with nitrogen, silanized with 3-mercaptopropyl trimethoxysilane in anhydrous toluene under nitrogen, incubated with 4-maleimidobutyric acid N-hydroxysuccinimide ester (GMBS) (Sigma-Aldrich, St. Louis, MO), and then
treated with NeutrAvidin. The captured antibody was immo-
ibilized to the inner surface of the capillary by recirculating
biotinylated anti-E. coli O157 antibody (10 μg ml⁻¹ in PBS
containing 0.05% Tween-20; PBST) for 1 h, followed by in-
cubation with 1% BSA to block remaining reactive sites. Af-
ter rinsing with PBST, capillary tubes were dried by purging
with nitrogen gas and stored at 4 °C until use.

2.4. Signal measurement instrument

The optical bench for the capillary immunosensor is basi-
ically the same as reported by Ligler et al. (2002) on loan from
the Naval Research Laboratory to Creatv MicroTech (Fig. 1).
The lenses, optical filters, and the capillary holder are the only
components modified. The laser excitation source employed
here was a 635 nm, 12 mW diode laser (LaserMax, Bridge-
water, NJ). An optical chopper (315 Hz, Stanford Research Sys-
tems, Sunnyvale, CA). External reference input was provided
connected to a SR-510 lock-in amplifier (Stanford Research Sys-
tems). Biosensor capillary tubes with immobilized anti-O157
antibody were incubated statically with different E. coli O157
concentrations (10³, 10⁴ and 10⁵ cells ml⁻¹ in PBS, n = 5) at
ambient temperature for 1 h, then rinsed with PBST. Capture
efficiency was estimated using in vitro lysis of captured cells
with 1% Triton X-100 in PBS (37 °C for 10 min) followed
by quantitative PCR. Five microtiter plates of lysis solution
was used for real-time PCR performed with a Stratagene MX4000
(Stratagene, La Jolla, CA). A calibration curve was first con-
structed using a 10-fold dilution series of the plasmid stan-
dard containing the lacZ gene (10⁶ to 10⁷ copies μl⁻¹). There
was a strong linear inverse relationship (R² = 0.9997) between
threshold cycle (CT) and the log₁₀ number of lacZ copies
over 7 orders of magnitude (Fig. 2). The equation describ-
ing the relationship is CT = -3.2426 × log₁₀(lacZ) + 36.879.
The capture efficiency as determined by PCR was 23, 26 and
28% (mean = 26%) for input concentrations of 10³, 10⁴ and
10⁵ E. coli O157 cells ml⁻¹, respectively.

2.5. Real-time PCRs

The sequences of primers and probe for real-time PCR am-
plication of the lacZ gene have been previously described
(Higgins et al., 2003). The primers and probe were synthe-
sized by Sigma-Genosys (Houston, TX). The reporter dye
FAM (6-carboxyfluorescein) was conjugated at the 5’-end
of the probe, and quencher dye, Black Hole quencher (BHQ) dye
I was conjugated at the 3’-end. PCR mixture was prepared
in a 50-μl volume containing 5 μl of genomic DNA template
recovered from capillaries or standard DNA, 0.3 μM each
primer, 0.1 μM probe, 0.1 μM reference dye ROX, and 25 μl
of Brilliant® QPCR Master Mix (Stratagene, La Jolla, CA).
The reaction mixture was dispensed into thin-wall PCR tubes
and covered with optically clear caps (Stratagene). PCR was
performed with the Stratagene MX4000 thermal cycler at the
following cycle conditions: denaturation and enzyme activa-
tion at 95 °C for 10 min and 40 cycles of 95 °C for 30 s and
60 °C for 1 min, followed by a 5-min extension at 72 °C and
holding at 4 °C.

3. Results

3.1. Biosensor capture efficiency

Quantitative real-time PCR was used to determine the cap-
ture efficiency for O157 cells by the antibody-coated capil-
laries. Biosensor capillary tubes with immobilized anti-O157
antibody were incubated statically with different E. coli O157
concentrations (10³, 10⁴ and 10⁵ cells ml⁻¹ in PBS, n = 5) at
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The capture efficiency as determined by PCR was 23, 26 and
28% (mean = 26%) for input concentrations of 10³, 10⁴ and
10⁵ E. coli O157 cells ml⁻¹, respectively.

3.2. Biosensor sandwich assay

The capillary tubes were incubated statically with different
E. coli O157 concentrations (10³ to 10⁵ cells ml⁻¹ in PBS) at
ambient temperature for 1 h. After rinsing with PBST, tubes
were incubated with 10 μg ml⁻¹ of Cy5-labeled polyclonal
anti-O157 antibody for 1 h. After rinsing with PBST, fluores-
cence signals were measured using the integrating waveguide
biosensor. Based on the negative control (53.7 ± 5.4 μV),
the threshold detection value (average + 3S.D.) was set at
69.9 μV. The input concentration of 10⁵ ml⁻¹ E. coli O157
resulted in a significantly higher signal (94.9 ± 15.7 μV) than
that of the negative control (Fig. 3a).

Based on the capillary volume and the capture efficiency,
the signal per E. coli O157 cell was estimated according to the
formula: cells ml⁻¹ (input concentration) × 26% (capture ef-
ciciency) × 0.075 ml (capillary volume) × 2/3 (ratio of laser
excitation area to capillary tube length). The fluorescence sig-
nals for estimated E. coli O157 number are shown in Fig. 3a.
As few as 13 E. coli O157 cells per capillary were able to
generate a sufficient signal for biosensor detection (94.9 μV).
Thus, the biosensor detection limit for E. coli O157 appears
to be ≤10 cells with an assay time of ca. 3 h. There was a lin-
ear relationship (R² = 0.987) between the logs of fluorescence
signal and cells captured, allowing for quantitative detection
(Fig. 3b).
3.3. E. coli O157 growth curve in biosensor capillary

Initial growth curves were conducted to assess the effect of volume on growth rate. Capillary (0.075 ml), or test tubes (10 ml) containing ca. $10^4$ E. coli O157 cells ml$^{-1}$ were filled with MLB-Y medium and test tubes subsampled and capillary tubes sacrificed with time ($n = 3$). Growth curves in capillary tubes and standard test tubes were essentially identical (Fig. 4 a). Therefore, the configuration and volume did not affect cell growth.

To evaluate growth of antibody-immobilized bacteria, capillaries with bound E. coli O157 cells (ca. 250 per capillary) were rinsed with PBST and filled with growth medium. At each time point, suspended cells and immobilized cells (cells captured after binary fission) were determined by quantitative real-time PCR. The bacterial growth of suspended cells was also determined by plating and colony counting.

Fig. 4b shows growth curves of suspended cells in capillary tubes and antibody-immobilized bacteria bound to the capillary surface. Growth curves for suspended cells using real-time PCR and plating and colony counting were essentially identical (not shown). The immobilized cells on the capillary surface (ca. 14,300) were saturated in 3 h, suggesting that this is the maximum capacity of the capillary. Clearly, the antibody binding of cells to capillary tubes had no adverse impact on subsequent exponential growth. The majority of bacterial cells were in the suspended portion (>96% in 3 h and >99% in 4 h), indicating that the growth medium containing cells from the capillary enrichment procedure was suitable for further genetic or antibiotic assays (Fig. 4 b).

4. Discussion

A variety of immunological methods have been described for the capture and detection of E. coli O157:H7 using solid supports such as magnetic beads, glass beads, filters, dipsticks, and other materials (Chapman et al., 1997; Czajka
Fig. 4. (a) Growth curve of non-immobilized E. coli O157 in the biosensor capillaries (1.66 x 75 mm) and test tubes (15 x 125 mm), three replicates per time point. (b) Growth curve of antibody-immobilized E. coli O157 in the biosensor capillary. “Suspended” indicates bacterial cells detected in the medium. “Immobilized” indicates cells captured and re-captured on the capillary surface.

5. Conclusion

A novel approach is described for the detection of water-borne pathogens, combining the integrating waveguide biosensor with multiple assays, which allows for determination of bacterial serotype, genotype, and viability. The data presented here demonstrate the concept that the biosensor system is capable of directly capturing E. coli O157 from water with subsequent detection in a fluorescence sandwich assay and quantitative real-time PCR. This system can potentially be adapted for the detection of other food- and water-borne pathogens.

Acknowledgements

We thank Dr. Francis Ligler (Naval Research Laboratory, Washington, DC) for helpful advice and comments. We thank Valerie McPhatter (USDA/ARS, Environmental Microbial Safety Laboratory) for technical assistance. This work is supported by grant R43 AI052684 from the National Institutes of Health and a grant from the Maryland Technology Development Corporation.

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