Sensing and Bio-Sensing Research 6 (2015) 59–66

Contents lists available at ScienceDirect

Sensing and Bio-Sensing Research

journal homepage: www.elsevier.com/locate/sbsr

Fast and sensitive detection of bacteria from a water droplet by means of electric field effects and micro-Raman spectroscopy

David S. Liao, Joshua Raveendran, Shiva Golchi, Aristides Docoslis *

Department of Chemical Engineering, Queen's University, Kingston, ON K7L 3N6, Canada

ARTICLE INFO

Article history:
Received 27 July 2015
Received in revised form 15 September 2015
Accepted 17 September 2015

Keywords:
Raman spectroscopy
E. coli
Biosensor
Dielectrophoresis
Electroosmosis
Bacterial detection in water

ABSTRACT

A surface-based method that can provide early and label-free detection of target microorganisms in water is presented. The method combines three key features, namely, (a) accelerated transportation to, and focusing of bacteria on, the detection surface by means of alternating current (AC) electrokinetic effects, (b) selective capture of the target microorganism using surface-immobilized antibodies, and (c) sensitive detection of target bacteria with micro-Raman spectroscopy. The non-uniform electric field is created by an AC signal-driven planar, quadrupolar, gold microelectrode array that is deposited on the detection surface (oxidized silicon wafer). AC electroosmosis and dielectrophoresis combine to produce the desired concentration amplification of bacteria on the capture surface within a few minutes. Bacterial detection is, subsequently, accomplished through Raman spectra acquired at pre-determined locations near the electrodes after sample removal and rinsing of the capture surface. Using this technique, detection of *Escherichia coli* K12 (target microorganism) at concentrations as low as 10^2 bacteria per mL was reproducibly achieved from 50 μL sample droplets. This method also permits the selective retention of a target microorganism from polymicrobial mixtures, as demonstrated here with the capture of *E. coli* K12 from their mixtures with *Micrococcus luteus*.

© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Current drinking water bacteria tests are performed in a laboratory, or use microbiological culture kits that require a minimum of 18–24 h of incubation [1]. Microfluidics-based prototypes demonstrating reduced testing time have been recently reported for field monitoring of bacteria [2,3]; however, they still have relatively high detection limits (~10^4 cell/ml), low sample throughput and may require expensive supporting equipment. Molecular diagnostic detection methods (DNA or RNA-based) are still laboratory-based [4,5]. The ability to concentrate bacteria *in-situ* without culturing, or with significantly reduced culture times, is a unique competitive advantage for new technologies and key toward addressing challenges faced with bacterial culturing and detection. One way to achieve desired concentration amplification in microfluidic settings is by means of specially designed microelectrode arrays that can cause electric field effects, such as electroosmosis and dielectrophoresis [6]. Proof-of-principle demonstrations of how electric-field-assisted preconcentration strategies enable the detection of dilute target analytes are numerous [7,8]. Some characteristic examples are cited here for viruses [9,10], bacteria and yeast [11,12], DNA [13], and peptides [14].

For a successful biosensing application, concentration amplification must be paired with sensitive signal transduction. Micro-Raman spectroscopy, *i.e.*, Raman spectroscopy performed through microscope optics, is rapidly emerging as a promising *in situ* bacterial detection and characterization technique that can be readily interfaced with microfluidic detection platforms [15–17]. A comprehensive review on this subject can be found in [18]. Although very few reports currently exist on bacterial detection methods that combine micro-Raman spectroscopy with electric field-assisted pre-concentration, the results are already very impressive. Specifically, using dielectrophoretic focusing and micro-Raman spectroscopy, Schröder et al. demonstrated a method to classify pathogenic bacteria from urine samples (>10^5 cfu/mL) within a few minutes and without labeling [19]. Using a microelectrode array Cheng et al. demonstrated bacterial focusing and detection by means of surface enhanced Raman spectroscopy (SERS) on a roughened metal surface inside a microfluidic chip [20]. Recently, Madiyar et al. performed SERS on nanotag-labeled bacteria concentrated by means of dielectrophoresis over a nanoelectrode array and were able to demonstrate a detection limit of app. 10^2 cfu/mL

* Corresponding author.
E-mail address: docoslis@queensu.ca (A. Docoslis).

http://dx.doi.org/10.1016/j.sbsr.2015.09.005
2214-1804/© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

in complex matrices (e.g., chicken broth) [21]. SERS is a powerful detection and characterization method, which can amplify a Raman signal by a thousand-fold or more; however, it usually involves nanoparticles or specially prepared substrates that result in more complicated detection protocols.

Here we demonstrate a simple, label-free, surface-based method that employs micro-Raman spectroscopy for the detection of bacteria from sample droplets of only a few microliters without need for signal amplification. To increase the speed and efficiency of detection, a planar microelectrode array is incorporated into the detection surface. The array generates an alternating current (AC) electric field that causes accelerated bacterial transport from the bulk of the droplet to predetermined concentration spots on the sensor's surface. Moreover, decoration of the microelectrode area with antibodies adds selectivity to the method, thus permitting the detection of a target pathogen from samples containing mixed bacterial populations.

2. Materials and methods

2.1. Materials

Biotin-conjugated polyclonal antibodies to *Escherichia coli* K12 were purchased from Cedarlane (Burlington, ON). All other materials were purchased from Sigma–Aldrich Canada Co. (Oakville, ON). Polished silicon wafers (4″ diameter) with a thermally grown SiO_2 layer (0.5 μm) were purchased from University Wafer (South Boston, MA, USA). Millipore® water (18.2 MΩ cm) was used throughout the experiments.

2.2. Microchip fabrication

The negative photoresist ma-N 1405 (Microresist Technologies GmbH, Berlin, Germany) was used to photolithographically transfer the microelectrode pattern from a chromium mask to the silicon substrate. A 5 nm layer of thermally evaporated chrome was used to improve the adhesion of the deposited Au layer (100 nm thickness) to the SiO_2 substrate.

2.3. Microchip functionalization

A 15 μL droplet (500 μg/mL) of biotinylated bovine serum albumin (BSA) dissolved in phosphate buffer saline (PBS) was placed over the microelectrodes and left overnight for BSA adsorption to occur. After washing with PBS (15 min) and water (15 min) and drying in a stream of air, a 15 μL droplet (2.5 mg/mL) of avidin solution was placed on the chip for 2 h. After washing and drying, a 15 μL droplet (4 mg/mL) of biotinylated antibody solution was placed on the chip and left for 3 h. Functionalization occurred in high humidity environment to prevent droplet evaporation. All chips were used immediately after functionalization.

2.4. Sample preparation

E. coli K12 and *Micrococcus luteus* were grown on LB agar plates. Low conductivity suspensions (1.0 ± 0.5 mS/m) were created by suspending the bacteria in water, centrifuging at 5800 rpm for 10 min and repeating the suspension/centrifugation step twice. The concentration of the stock suspension was determined with the use of a Petroff-Hausser bacteria counter. Lower concentration samples were prepared with serial dilutions from the stock and used within a few hours.

2.5. Dielectrophoretic bacterial capture

All experiments were carried out at room temperature. The bacteria suspensions were used immediately after dilution. The microelectrode chip was placed on a custom-made holder, which was mounted on a motorized microscope stage. Power to the microelectrodes was supplied by a signal generator (BK Precision 4040A). The microelectrodes were connected to the source so that a phase difference of 180° existed between adjacent electrodes. The value of applied voltage and frequency was monitored by an oscilloscope (Tektronix 465). A 50 μL drop of the bacteria suspension was placed over the microelectrode center using a micropipette (Fig. 1a). The collection was run for 15 min at 10 kHz and 12 V_{pp} (peak-to-peak). After collection, the chips were washed with PBS for 15 min, rinsed with water and dried in a stream of air. Images of the captured bacteria were taken with a CCD camera (Lumera, Infinity 3) coupled to an optical microscope (Olympus, BX-41).

2.6. SEM sample preparation

Collected bacteria prepared for SEM visualization through a procedure adapted from previous work [22]. The chip containing the captured bacteria was immersed in a 3% (v/v) glutaraldehyde solution in 0.1 M phosphate buffer saline (PBS) for 24 h. The chip was then rinsed in PBS for 15 min. Cell dehydration was accomplished by sequentially submerging the chip for 10 min each time in a series of ethanol solutions in water in the following order: 10%, 30%, 50%, 70%, 90% and 100%. After critical point drying and gold-coating, the surface of the chip was ready for SEM examination.

2.7. Raman measurements

A HORIBA Jobin Yvon micro-Raman Spectrometer (Model: LabRAM) with a 632.8 nm He/Ne laser (17 mW), 1800 1/nm grating and an Olympus BX-41 microscope system were used. The collection of spectra was performed in the backscattered mode under the following conditions: ×100 microscope objective, 500 μm pinhole, 500 μm slit width. To ensure consistency across measurements, the laser beam was focused on the substrate so that it maximized the silicon peak intensity at 521 cm^{-1}.

2.7.1. Point measurements

Raman spectra were acquired from a total of twelve spots, i.e., three spots per microelectrode gap (Fig. 1b). The spots were located along the line of minimum microelectrode separation: two at the electrodes' edge and one midway in the gap. Each spectrum represents the average of two measurements. After acquisition, the spectra were subjected to blank spectrum subtraction and baseline correction.

2.7.2. PCA analysis

Principle component analysis (PCA) is a multivariate statistical technique which generates a set of uncorrelated indices from a linear combination of the original variables. The indices or principal components are ordered by decreasing variance. Thus later principal components can potentially become negligible. When the original set of variables are correlated, later principal components can be discarded, reducing the number of variables required to accurately describe the data. A total of seventy-two reference Raman spectra were obtained; twenty-four of *E. coli*, twenty-four of *M. luteus* and twenty-four of a silicon surface functionalized with BSA, avidin and biotinylated antibodies. The Raman spectra were acquired at the previous conditions but with a slit width of 600 μm. The acquisition window was set from 1150 to 1850 cm^{-1}. An acquisition time of 20 s and 3 averages were used for each spectrum. The spectra were processed using Matlab 7.10.0 (R2010a). A Savitzky-Golay FIR smoothing filter (polynomial order 2 and frame size 31) was applied

Download English Version:

https://daneshyari.com/en/article/804037

Download Persian Version:

https://daneshyari.com/article/804037

Daneshyari.com