On-chip Mach-Zehnder Interferometers for rapid detection of bacteria in drinking water

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Abstract

A miniaturized optical immunosensor for the simultaneous label-free determination of bacteria in drinking water is presented. The sensor consists of an array of ten Mach-Zehnder interferometers (MZIs) integrated on silicon chip along with their corresponding broad-band light sources. The transmitted spectra of the MZIs were continuously recorded through a spectrometer. The spectral shifts caused by changes of the effective refractive index on the sensor surface due to bioreaction were converted to phase shifts through discrete Fourier transform. For the analysis, the different MZIs of the chip were biofunctionalized with the respective S. typhimiurim and E. coli membrane antigens. Then, mixtures of bacteria solutions with anti-bacteria antibodies were pumped over the chip followed by reaction with biotinylated anti-species specific antibody and streptavidin. The assays were fast (10 min), sensitive (LODs <$2X10^2$ CFU/mL), accurate (recovery 86-115%), repeatable with intra- and inter-assay CVs <5% and 8%, respectively, and the chip could be regenerated/reused for at least 20 times. Considering the low detection limits achieved in combination with the short analysis time and the small chip size, the proposed immunosensor could find wide application for bacteria detection in drinking water at the point-of-need.

Keywords: immunosensor, bacteria, drinking water, Mach-Zehnder Interferometers

1. Introduction

The contamination of drinking water by pathogenic bacteria, such as E. coli, Vibrio cholera, Salmonella spp. and Shigella is a global health alarm. According to WHO, 5 million deaths are associated to water related diseases annually (Cabral et al., 2010). For this reason, the assessment of drinking water contamination by bacteria is of crucial importance to protect consumer's health. The conventional methods for bacteria detection are based on culturing and plating, which are reliable but rather time-consuming. In order to shorten the analysis time, ELISA- and DNA-based methods have been employed for bacteria identification (Shabani et al., 2015). However, the above methods require skilled personnel and cannot be performed at the point-of-need. Recently, biosensors based on electrochemical, piezoelectric or optical transducers are gaining ground in waterborne bacteria

detection (Li et al., 2014; Zhang et al., 2016; Guo et al., 2012). Although these sensors claim inexpensive analysis and potential for miniaturization, they lack in sensitivity and often require labels for signal enhancement to improve detection limits.

In this report, for the first time, we investigated the potential of an optical biosensor based on an array of ten Mach-Zehnder interferometers (MZI) integrated along with their corresponding light sources on silicon chips (Angelopoulou et al., 2015; Angelopoulou et al., 2018) for the simultaneous and sensitive detection of S. typhimiurim and E. coli in drinking water.

2. Experimental

2.1. Reagents, chip fabrication and signal processing

The rabbit polyclonal anti-S. typhimiurim LPS antibody was from Bio-Rad (UK) and the mouse monoclonal anti-E. coli LPS antibody was from Origene (Germany). E. coli LPS was from Creative Diagnostics (USA). APTES, bovine serum albumin (BSA) and S. typhimiurim LPS were from Sigma-Aldrich (Germany).

The chips are fabricated following mainstream silicon technology as described previously (Angelopoulou et al., 2015; Angelopoulou et al., 2018). The ten MZIs converge at the edge of the chip where an external a miniaturized spectrometer is positioned (QE65000, Ocean Optics). During the assay, the spectra of the MZIs were continuously recorded and the spectral shifts caused due to bioreaction were converted to phase shifts (analytical signal) through discrete Fourier transform.

2.2. Chemical and biological activation of the chip

The chips were cleaned/hydrophylized through O_2 plasma treatment for 30 s and immersed for 2 min in a 0.5% (v/v) APTES solution. Then, the chips were washed, dried, and heated at 120 °C for 20 min. After that, 3 MZIs per chip were spotted with 200 µg/mL of *S. typhimurium* LPS solution, 4 MZIs with 200 µg/mL of E. coli LPS solution, and the remaining 3 with 100 µg/mL BSA solution (non-specific binding) using the BioOdyssey Calligrapher Mini Arrayer. After spotting completion, the chips were washed, blocked for 2 h with 1% (w/v) BSA in 0.1 M

NaHCO$_3$ solution, washed again and dried under nitrogen stream.

2.3. Assay for the detection of bacteria in drinking water

A microfluidic module was attached onto the chip for the delivery of the samples and the chip was positioned in the docking station of the measuring apparatus. In parallel, calibrators/samples were mixed at 1:1 volume ratio with the anti-LPS polyclonal and monoclonal antibodies against *S. typhimurium* and *E. coli,* respectively, and incubated for 60 min. For the immunoassay, 200 μL of these mixtures were run over the chip at a rate of 35 μL/min, followed by 100 μL of biotinylated anti-species specific antibodies and 100 μL of streptavidin solution. After that, 100 μL of a 0.1 M HCl solution were passed over the chip so as to regenerate the surface prior to run the next sample.

3.   Results

3.1. Optimization of assay parameters

For the detection of bacteria using the MZI chip, all assay parameters including the concentration of the LPS solutions used for coating, the concentration of anti-bacteria LPS specific antibody, of anti-species specific antibodies and streptavidin, as well as the composition of all the buffers used have been optimized with respect to absolute signal and assay sensitivity.

As shown in Fig. 1, a 3-step assay configuration was employed involving 5 min reaction with the anti-LPS antibodies, followed by 2.5 min reaction with anti-species specific antibodies, and 2.5 min reaction with streptavidin (total assay time 10 min). Following this protocol zero calibrator signals > 1 rad were obtained for both bacteria.
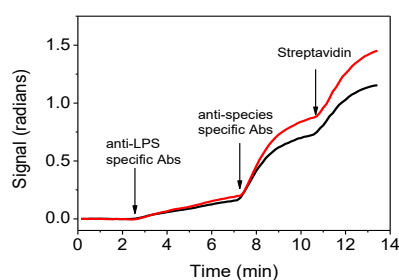

Figure 1. Real-time signal corresponding to zero calibrators (0 CFU/mL) of S. typhimiurim (red line) and E. coli (black line).

3.2. Analytical characteristics of the MZI chip

The linear working range for both assays ranged from 10^3 to 10^7 CFU/mL. In Fig. 2, calibration curve for *S. typhimurium* is provided indicatively. The assays detection limit was determined as the concentration corresponding to signal equal to -3SD of the mean zero calibrator signals (21 replicate values from 3 chips; 7 MZIs per chip) and was 2×10^2 CFU/mL. The assays were accurate with recovery values in drinking water samples ranging from 86 to 115%, and repeatable with intra- and inter-assay CVs <5% and 8%, respectively. Moreover, the chip could be regenerated/reused for at least 20 times. The values determined for spiked drinking water samples

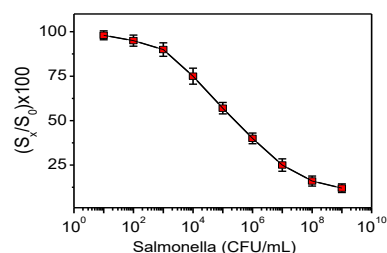with the immunosensor developed were in good agreement with those obtained employing in-house developed ELISAs.

Figure 2. Calibration curve of *S. typhimurium.*

4.   Conclusions

A miniaturized immunosensor for the simultaneous detection of S. typhimurium and E. coli in drinking water was developed. The chip provided label-free and real-time detection of the two bacteria. The LODs obtained for both bacteria, the short analysis time (10 min) and the small size of the chip make the proposed immunosensor ideal for on-site bacteria detection in drinking water samples.

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