Microfluidic-based Biosensor Chip for Rapid and Calibration-free Detection of Viable E. coli and Total Coliforms for Water Quality Control

Basic Information

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There are no publications.

Proposal Title: Microfluidic-based Biosensor Chip for Rapid and Calibration-free Detection of Viable *E. coli* and Total Coliforms for Water Quality Control

Principle Investigators:

Yu Lei, Associate Professor, Chemical & Biomolecular Engineering, University of Connecticut, Storrs, CT 06269-3222. Telephone: 860-486- 4554, Email: ylei@engr.uconn.edu

TaiHsi Fan, Associate Professor, Mechanical Engineering, University of Connecticut, Storrs, CT 06269-3222. Telephone: 860-486- 0553, Email: thfan @engr.uconn.edu

Introduction/Research Objective:

Waterborne microbiological contaminations remain one of the major threats to public health. The Centers for Disease Control and Prevention has reported that each year, 4 billion episodes of diarrhea result in an estimated 2 million deaths, mostly among children. Waterborne bacterial infections may account for as many as half of these episodes and deaths.

In the past decades, a variety of technologies have been developed to detect the total coliforms and *E. coli* in drinking water. However, they usually take 18-24 h to complete. From a public health standpoint, it is too time-consuming to announce a boil water notification if the sample is positive for total coliforms or *E. coli*. Therefore, an innovative, calibration-free, easy-operation, robust, and ultrasensitive method for fast-screening skeptical drinking water samples is highly demanded. Preferably, it can also discriminate the viability of total coliforms and *E. coli* as conventional EPA-approved methods.

The research objective of this multidisciplinary proposal aims to develop a novel, cost-effective and user-friendly microfluidic-based digital biosensor chip (in conjunction with a commercial available large-volume water sample concentrator) for rapid, ultra-sensitive, and calibration-free detection of viable *E. coli* and total coliforms in drinking water, based on the activity of β-glucuronidase for *E. coli* and β-galactosidase for total coliforms, respectively. A number of novel features are introduced to the proposed system to make the MEMS biosensor faster and more

sensitive toward the targets. This project will also positively impact education of graduate, undergraduate and high school students by integrating advanced water quality monitoring into their educational and laboratory training.

Methods/Procedures/Progress:

1. Completing the training needed for the fabrication of the proposed microfluidic devices at Center for Nanoscale Systems (CNF) in Harvard University

The student received extensive training for the total of 18 training sessions required for our device fabrication at Harvard CNF over 6 months, including safety training, Nexx PECVD, Suss MJB4 Mask Aligner, Cleanroom Headway Spinner Training, Technics Plasma Stripper/Cleaner, Anatech Barrel Plasma System, Tystar Bank2 Wet/Dry Oxidation, Tystar Bank2 TEOS Silicon Dioxide, Tystar Bank2 Metal Anneal, Tystar Bank1 Silicon Nitride, Tystar Bank1 Polysilicon, Tystar Bank1 Non-Metal Anneal, STS PECVD, Denton E-Beam Evaporator, South Bay RIE, Veeco Dektak Profilometer, Scanning Ellipsometer, and Nexx RIE.

2. *Fabrication of microfluidic device*

Microchannels with the narrowest cross-section feature of 10 μm in width and 15-25 μm in height were fabricated in PDMS by the standard soft-lithography technology, developed in our previous research. Figure 1 shows the as-prepared microfluidic device with patterned electrochemical sensing electrodes and microfluidic channel and fluidic connectors developed in PI laboratory. In principle, arbitrary topology, depth, width, and feature size ranging from several microns to hundred microns can be fabricated. In brief, SU-8 2025 and 2015 negative resists (Microchem) were spin-coated on 4" silicon wafers. Exposure with the mask and development with propylene glycol methyl ether acetate (PGMEA) produced channels with the pre-designed feature size. Polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) was mixed at a 10:1 ratio and poured over the SU-8 mold which was then baked at 80 °C for 1.5 hr to create the final channel. On the

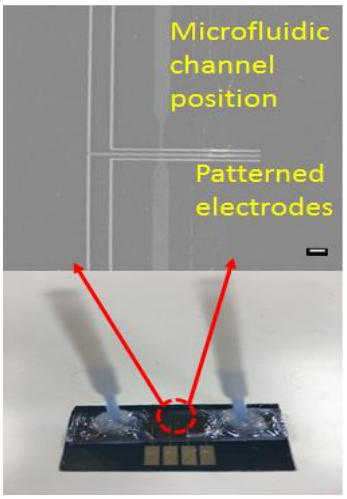

Figure 1. The as-prepared microfluidic device with patterned electrochemical sensing electrodes. Scale bar = 100 μm.

other hand, the electrochemical sensing electrode patterns on Si wafer are fabricated in photoresists with photolithography and then Au/Ti metal layers were sequentially deposited on silicon wafer using thermal evaporation technique. After lift-off process in the photoresist developer, the electrodes with pre-designed shapes and dimensions were formed on the substrate surface. To complete the device fabrication, the PDMS channel was plasma bonded to the Si substrate containing the pre-patterned electrodes and then assembled with appropriate connectors to form a microfluidic system. By regulating the perfusion rate of the carrying electrolyte, the targeted bacteria can be deployed to the channel for the proposed detection.

Results/Significance:

***1. E. coli* culturing**

As *E. coli* possesses both activity of β-glucuronidase (unique for *E. coli*) and β-galactosidase (ubiquitous for total coliforms), *E. coli* will be used in this study to represent both *E.coli* and total coliform. First, a safe *E. coli* lab strain (DH5α), obtained from the strain collection of our laboratory, was used as a model bacterium for the training purpose. *E. coli* was inoculated into Luria broth (LB) medium and incubated overnight on a gyratory incubator shaker at 37 °C and 200 rpm, which allowed the growing stationary phase to be reached. Then, bacterial cultures were serially diluted (10-fold steps), and 10 μL aliquots of samples were applied to LB agar plates and incubated for 24 h at 37 °C, for enumeration of colonies. At the same time, the stationary-phase cultures were diluted to different concentration ranging from 1 cfu/mL to 10^6 cfu/mL in buffer.

***2. Direct detection of E. coli* in microfluidic-device.**

As a preliminary test, the amperometric counting was conducted using the developed device. A constant voltage (+0.6 V) is supplied through the modified working electrode (deposition of Ag on the patterned electrodes to shrink the cross-section area channel at the position of electrodes) and the current vs time response was reordered for the flow of carrying buffer solution in the absence and presence of *E. coli.* The solution was driven by syringe pump

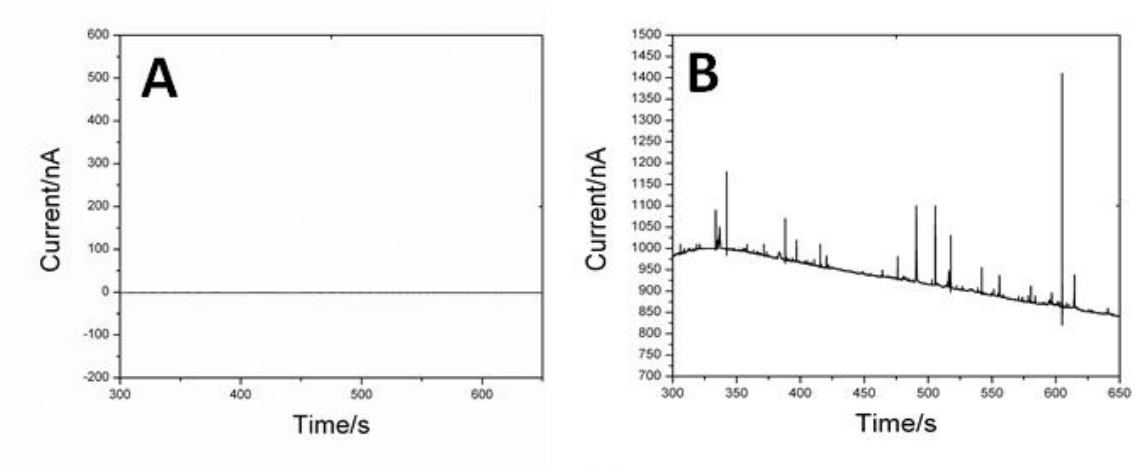

Figure 2. The preliminary study of ameprometric detection for PBS buffer sample in the absence (A) and presence (B) of E. coli.

to flow through the microfluidic device. Figure 2 shows the corresponding results. One can see that a lot of pulses are recorded in the presence of *E. coli*, while only background noise was observed for the buffer in the absence of *E. coli*. It is hypothesized that each pulse may be resulted from the pass of one *E. coli*. This result indicates that it is highly possible for direct counting of *E. coli* without using any calibration curve, which will be further investigated in the 2nd year.

3. Design of microfluidic device with two electrochemical sensors

To directly amperometric counting of both *E. coli* and total coliforms, microfluidic device with two patterned electrochemical sensors was designed. Figure 3 show the design of two patterned electrochemical sensors after photoresist development. Briefly, P-type Boron doped, <1 0 0> orientation silicon wafer are used. Silicon wafer was dry-oxidized through CVD-10 with an oxide layer of 106 nm in thickness (measured by ES-2 Scanning Ellipsometer). Positive photoresist Shipley 1805 was spun at 4500 rpm to create a 5 micron thickness of photoresist onto LOR3A which was first spun at 3500 rpm to create roughly 320 nm thickness of LOR3A on the wafer, followed by UV light exposure at wavelength of 405nm for optimized intensity at 52 mJ/cm^2 with a focus at -2 plane (Defocus) by using Maskless Aligner MLA 150. The treated substrate was developed in CD-26 developer for 1 minute and dried by nitrogen gun. The detailed feature of electrodes pattern can be referred to Figure 3. We will continue to do the metal deposition, followed by lift-off to generate the two sensors on the Si wafer, thus realizing simultaneously detection and differentiation of *E. coli* and total coliforms. The new device will be employed in the 2nd year research.

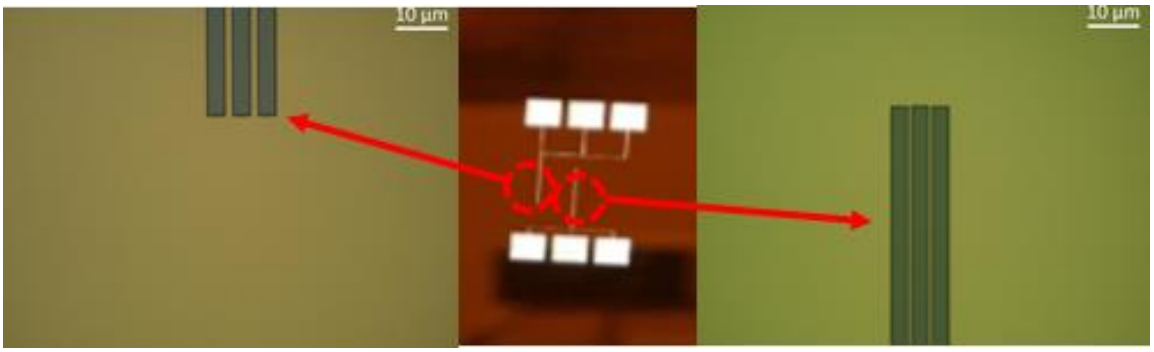

Figure 3. Featured pattern of pre-designed microelectrode devices (scale bar: 10 □m) after UV light exposure at 405 nm by Maskless aligner (MLA) 150.