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Bacteria detection based on its blockage effect on silicon nanopore array

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ABSTRACT

Bacteria detection plays an important role in the guarantee of food and water safety. This work proposed a new sensing strategy for the rapid detection of bacteria based on its blockage effect on nanopore array, which was prepared from electrochemically etched silicon. With the assistance of microfluidic technology, the nanopore array attached with *Escherichia coli* antibody can selectively and rapidly capture *E. coli* bacteria, resulting in the decrease of pore accessibility. The signal of pore blockage can be measured by in-direct Fourier Transformed Reflectometric Interference Spectroscopy (FT-RIS). The pore blockage signal has a linear relationship with the logarithm of bacterial density in aqueous sample within the range from 10^3 to 10^7 cfu ml^{-1}. Due to the specific interaction between the antibody and target bacteria, only the *E. coli* sample displayed significant pore blockage effect, whereas the non-target bacteria, *Nox* and *P17*, almost did not show any pore blockage effect. The strategy established in this work might be pervasively applied in the rapid detection of target bacteria and cell in a label-free manner.

1. Introduction

Nanopore technology has emerged as a promising sensing technique within the past 20 years as it has the potential to sequence DNA strands at high speed and low cost (Deamer and Branton, 2002; Iqbal et al., 2007; Venkatesan et al., 2009). The original concept of nanopore sensing is based on the detection of ionic pore current when individual molecules pass through a single nanopore (Deamer and Branton, 2002). The range of analytes that can be detected with nanopores now expands to small molecules, organic polymers (Krasilnikov et al., 2006), peptides (Zhao et al., 2009), proteins (Fologea et al., 2007), enzymes (Kukwikila and Howorka, 2015), and biomolecular complexes. In the meantime, a variety of material such as silicon nitride, silicon dioxide, silicon, silicate and organic polymers are now used for the construction of solid state nanopore sensor. Up to now, most of nanopore sensor are targeting to the single molecular analysis on a single nanopore platform. While the single nanopore sensor display high sensitivity for molecular detection, its fabrication process is still difficult and inconvenient, which usually require a high cost manufacturing facility in a clean-room. Biosensor based on nanoporous membranes consisting of nanopore array have been reported for the detection of drugs, proteins, and oligonucleotides. For example, an ssDNA-modified porous anodic alumina membrane has been proposed for the label-free detection of interaction between DNA on the nanochannel surface (Li et al., 2010). The sensing strategy was based on the obstruction of nanopore array after the hybrid of complementary DNA strand. The blockage of nanopore array was probed by redox species, which can be detected by gold film electrochemical detector sputtered at the end of nanochannels. A simple label-free electrochemical biosensor based on screen-printed carbon electrode (SPCE) modified with alumina membrane has been developed for the detection of proteins (de la Escosura, 2010). Mesoporous silicon coated with gold layer was also exploited as a switchable electro-biosensor in our group (Feng and Wu, 2012). Screening of antisense oligonucleotides drug based on the switchable biosensor was demonstrated (Feng and Wu, 2012). Biosensor based on nanopore array is not designed to detect single molecule because the sensing signal come from the pore array is averaged. Despite its lower sensitivity compared with single nanopore sensor, the application of nanopore array is promising, since the nanopore array is easier to be available, and usually do not require a strict operational condition.

In this work, we demonstrated that optical sensor based on silicon nanopore array is a good platform for the detection of bacteria. The pore array on a membrane can be effectively blocked by bacteria, which was captured by antibody attached on the membrane surface. Usually, blockage of the nanopore can be probed by either measuring the electrochemical current of redox

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species or the migration current of electrolyte ions. However, for bacteria or cell analysis, small molecule probes may not be suitable, because the bacteria or cell captured by antibody cannot closely stick on the porous surface, leaving behind a sufficient space for admitting small molecular probes into the pore channel. To deal with the problem, high-molecular-weight (HMW) probes are needed. But electrochemical or electrical method is not the good choice to detect the signal of HMW probes. Therefore, it is of great importance to establish an optical method to measure the signal of pore blockage when HMW probes are used. Herein, electrochemically etched porous silicon (pSi) with ordered nanopore array was used as the sensing film, which was integrated into a microfluidic channel. HMW probes migrating into the nanopore layer can be sensitively detected by real time Fourier-Transformed Reflectometric Interference Spectroscopy (FT-RIS) due to the change of refractive index in the layer (Pacholski et al., 2005). If a partial of pore is blocked by target bacteria, the fraction of pore volume occupied by the probe molecules will thereby reduce. Consequently, the shift in effective optical thickness (EOT) caused by infiltrating of probe molecules will decrease compared with the EOT value measured on a fully opened nanopore array. Because the difference between the two EOT values is correlated with the density of bacteria occupied on the surface of nanopore array, the quantitative analysis of target bacteria can been achieved. This work might be the first using label-free optical method to report the pore blockage event caused by immuno-binding. The nanopore array sensing technology may find promising application in the detection of target bacteria and cell for environmental monitoring and clinical diagnosis.

2. Material and methods

2.1. Preparation of porous silicon

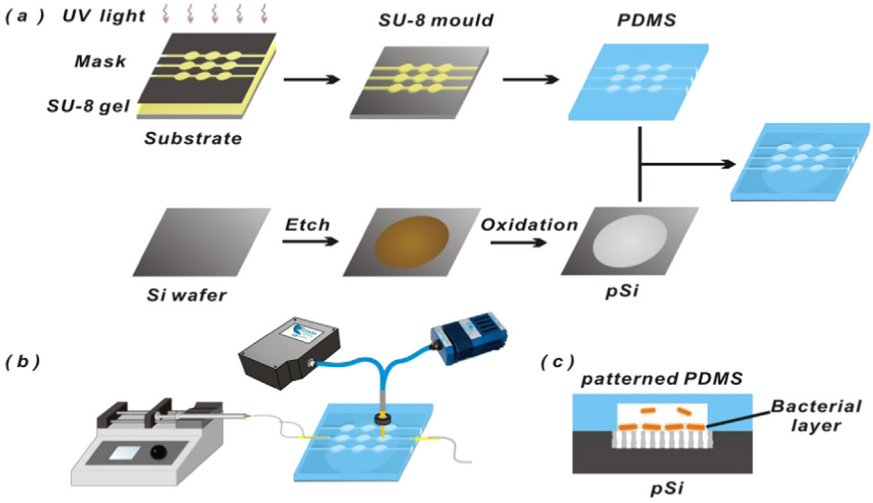
PSi was prepared by the electrochemical etching of single-crystal Si wafers in a two-electrode configuration using a platinum mesh counter-electrode. Si wafers (P++, 0.1 mΩ cm, Siltronix Co., France) with an exposed area of 1.2 cm^2 were contacted on the back side with a strip of aluminum foil and mounted in a Teflon etching cell. Electrochemical etching was performed at a constant current of 180 mA for 60 s in electrolyte mixture solution of HF (49%) and ethanol (3:1, v/v). An NJ-320 potentiostat/galvanostat (Najing Scientific Instrumental Co., Xiamen, China) was used as the current source. After etching, the pSi was thoroughly rinsed with ethanol and dried in a nitrogen gas flow. Then, the sample was thermally oxidized at 800 °C for 20 min. The porosity of oxidized porous Si film was determined from reflectance spectra using the Spectroscopic Liquid Infiltration Method (SLIM) (Segal et al., 2007).

2.2. Fabrication of PDMS microfluidic channel

The workflow for constructing a pSi-based microfluidic sensor is illustrated in Scheme 1a. Briefly, SU-8 mold was fabricated as a master template for casting of microfluidic structure onto a PDMS film using an UV photolithographic procedure. First, a layer of SU-8 (thickness ~400 μm) was spin-coated on a cleaned Silicon wafer. Then, the SU-8 film was covered with a photo mask whose pattern was designed with CorelDraw software (Corel Inc.) The film was exposed to ultraviolet light followed by a development procedure. PDMS (Sylgard 184, Dow Corning) was mixed with the as supplied cross-linking reagent in its standard volume ratio (10:1) and degassed at a vacuum of 0.88 bars for 45 min. The degassed PDMS was poured on the SU-8 mold and was degassed again for 30 min to remove bubbles. The wafer was baked at 70 °C for 1 h to completely crosslink the PDMS. At last, the PDMS film patterned with microchannel and holes was peeled slowly from the SU-8 master. The patterned PDMS film was sealed with the porous Si chip before both of them was treated by oxygen plasma for 30 s. The position of the channel on the PDMS film should be carefully aligned to the etched area of pSi chip. Finally, the patterned PDMS film was sealed with another PDMS film but without channels.

2.3. Attachment of E. coli antibody on the porous silicon chip

To activate the oxidized pSi surface, solution of 0.1 mol L^{-1} NaOH was loaded into the microchannel. After 30 min, the channel was washed with deionized water to remove the excess amount of NaOH. Then the microchannel was treated with 0.1 mol L^{-1} HCl for 30 min, and washed with deionized water. After the channel was dried with a stream of nitrogen gas, the pSi surface was reacted with 3% 3-aminopropyltriethoxysilane (APTES, ACROS Organics) of ethanol solution for 1.5 h. After the channel was washed with pure ethanol, the amino-terminated surface was then reacted with 2.5% glutaraldehyde (GA) of aqueous solution for 2.5 h. The GA activated surface was finally attached with *Escherichia coli* (*E. coli*) antibody by injecting the antibody solution (40 μg L^{-1}) into the microchannel, which was subsequently store at 4 °C overnight.



Scheme 1. (a) Procedures for assembling of microfluidic pSi biosensor. The test zone of etched porous silicon (pSi) chip was defined by a PDMS film patterned with microchannels (length, ~1.2 cm; width, ~500 μm; depth, ~250 μm) and microwells (diameter, ~2 mm; depth, ~250 μm). (b) The schematic experimental setup. (c) The schematic cross-sectional view of the microfluidic biosensor based on Si nanopore array.