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A cantilever biosensor exploiting electrokinetic capture to detect *Escherichia coli* in real time

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ABSTRACT

A cantilever biosensor with integrated electrodes for piezoelectric actuation and electrokinetic capture is used to detect cells in real time. The sandwich electrodes used for electrokinetic capture present advantages over side-by-side electrodes in terms of reducing the gap size between electrodes and the structural mass added to the cantilever. The 5th resonant mode (≈617 kHz) of the biosensor provided a signal-to-noise ratio of 82 within 10 min when *E. coli* was measured from a stagnant sample with a concentration of 10^7 cells/ml. The 7th resonant mode (≈1160 kHz) provided a signal-to-noise ratio of 26 within 10 min when *E. coli* was measured from a flowing sample (400 μl/min) with a concentration of 10^5 cells/ml. The estimated sensitivity of the 7th resonant mode is 326 fg/Hz based on images of captured cells.

1. Introduction

Miniature biosensors capable of detecting biological particles in real time possess great advantages over conventional laboratory tests which are time-consuming and costly. These biosensors find many applications in food and water analysis, clinical diagnosis, and environmental monitoring [1,2]. Several types of biosensors have been developed, including static and dynamic cantilever biosensors [3,4], amperometric and piezoresistive biosensors [5], and Raman spectroscopy biosensors [6,7]. Dynamic cantilever biosensors have enjoyed considerable attention due to their remarkable sensitivities (~fg/Hz) in air [8,9] and in water [10,11]. Such high sensitivities suggest that the mass of a small number of cells, even ones as small as *Escherichia coli* (*E. coli*) (≈1 pg), can be measured in both mediums, with water being the preferred medium because it is usually the natural medium of the biological target.

When a biological target is captured on the functionalized sensing surface of a cantilever, the mass of the cantilever increases and the resonant frequency decreases. For successful detection, the measurement system must provide a response with a high signal-to-noise ratio. To provide this, especially for samples with low concentrations, the cantilever biosensor must capture the biological target effectively and it must exhibit high sensitivity and low noise.

The biological target is typically captured using diffusion, sedimentation, or forced convection. To accelerate capture, electrokinetic effects can be used. Several electrode designs, including serpentine wires [12], dot electrodes [13], electrode rings [14], and quadrupolar electrode array [6], [15], have been reported. These electrodes create strong electric fields that induce bulk fluid motion through ac electroosmosis or electrothermal flow and capture biological targets through dielectrophoresis.

Besides accelerating the capture of the biological target, the sensitivity of the cantilever biosensor can be improved. In general, the sensitivity of a cantilever biosensor increases when its size decreases or when a higher order resonant mode is used [16]. By decreasing the size or by employing higher resonant modes to achieve higher sensitivity, the measurement noise is usually increased as well. Smaller cantilevers are subject to increased damping due to increased viscous effects [17], especially in water, and higher order resonant modes are usually harder to excite and measure accurately. Maraldo et al. demonstrated that the use of larger cantilevers (~mm) with geometric discontinuities and high order resonant modes [10,11] can improve sensitivity and reduce noise in water.

Inspired by our previous work [15,18] and the above literature, we developed a cantilever biosensor with integrated electrodes for piezoelectric actuation and electrokinetic capture. In this study we present and test this biosensor. To the best of our knowledge, this

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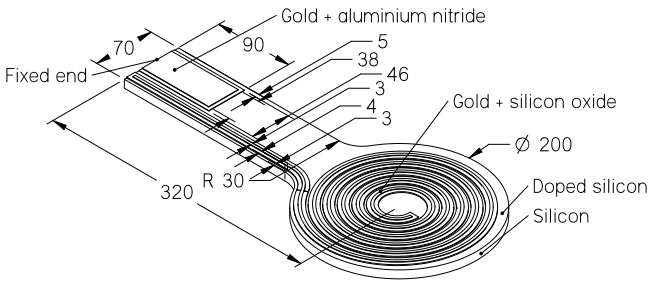

Fig. 1. Isometric drawing of the biosensor (dimensions are in μm).

is the first report of a cantilever biosensor exploiting electrokinetic capture to detect *E. coli* in real time.

2. Methodology

2.1. Design

The biosensor was fabricated with the PiezoMUMPs process [19], a process for micromachining piezoelectric devices in a silicon-on-insulator framework. The process has four layers: a silicon structural layer (10 μm) doped at the surface, a silicon oxide insulating layer (1 μm), an aluminium nitride piezoelectric layer (0.5 μm), and a gold electrode layer (0.52 μm).

The biosensor (see Fig. 1) consists of a microcantilever with three integrated electrodes. The first electrode is the doped silicon, the second electrode is the gold strip at the fixed end, and the third electrode is the gold strip that starts at the fixed end and forms a spiral at the free end. The piezoelectric strip is sandwiched between the doped silicon and the gold strip at the fixed end. Applying electrical potential across the piezoelectric strip causes it to deform and in turn causes the cantilever to vibrate primarily out of plane. The silicon oxide strip is sandwiched between the doped silicon and the other gold strip. Applying electrical potential across the silicon oxide strip creates a strong electric field, since the vertical and horizontal gap between the electrodes is small. At frequencies of ~100 kHz, the electric field induces ac electroosmotic flow over the biosensor and dielectrophoretic capture of cells on the edges of the electrodes (see our previous work [18] for more details about electrokinetic theory). Actuation and electrokinetic capture can be performed simultaneously by connecting the doped silicon to ground and connecting one gold strip to the actuation signal and the other gold strip to the electrokinetic capture signal.

Sandwich electrodes present advantages over conventional side-by-side electrodes for electrokinetic capture. The main advantage is that they use the underlying doped structure as one of the electrodes, thus reducing the electrode mass by half. This directly increases the sensitivity of the biosensor. Another advantage is that they allow the distance between electrodes to be reduced which results in strong electrical fields and stronger electrokinetic effects between the two electrodes. In general, in surface micromachining it is harder to reduce the space below 2 μm between two side-by-side electrodes due to mask alignment challenges, but it is easy to obtain submicron space for sandwich electrodes.

2.2. Dynamic response

The dynamic response of the biosensor was evaluated in air and water in order to determine which resonant modes should be used for testing. A periodic chirp signal with an amplitude of 3 V was applied across the piezoelectric film. A map of out-of-plane displacements in picometers (pm) was measured with a vibrometer (Polytech MSA-400 Micro System Analyzer) in the frequency range of 10–4000 kHz. This map provides the dynamic response at meshing points on the biosensor surface and the mode shape of the biosensor at any given frequency. Fig. 2a shows the dynamic response at the free end of the biosensor, and Fig. 2b shows the mode shape of the 5th and 7th resonant modes.

In air, 7 resonant modes have strong responses, with amplitudes exceeding 200 pm. The mode shapes (not shown) revealed that these modes are bending modes. In water, only 4 resonant modes have strong responses, with amplitudes exceeding 100 pm. The other three modes are observables but weak. The increased broadening and shifting of the resonant peaks from air to water is due to the increase in viscous effects [17]. The two highest order modes with the best response in water are the 5th mode (≈617 kHz) and 7th mode (≈1160 kHz). Both of these modes are bending modes and they have quality factors above 35. The mode order was confirmed with a modal analysis on COMSOL Multiphysics software.

2.3. E. coli *preparation*

A concentrated sample of *E. coli* K-12 was provided by Dr. Yang from the Department of Pathology and Molecular Medicine at Queen's University. The bacteria were killed via UV irradiation for 4 min. Diluted samples from 10^4 to 10^7 cells/ml were prepared with deionized water.

2.4. Chip preparation

A clean or previously used chip is taken for testing. Steel tweezers are used to move the chip during the different preparation steps. The chip is placed on a hot plate (Corning 6795-400D PC-400D) and heated from room temperature to 450 °C over 10 min in order to vaporizes any biological material on it [20,24]. The chip is removed from the hot plate, placed on a glass slide, and allowed to cool for 1 min. The chip is functionalized with poly-L-Lysine [21]. Since poly-L-Lysine is positively charged, it can be attached to negatively charged surfaces such as oxides and metals in water and it can electrostatically retain particles with negative surface charges such as *E. coli* [15,22,23]. A 100 μl droplet of Poly-L-Lysine solution (0.1% w/v H_2O) from Sigma-Aldrich is placed on the surface of the chip using a 10 μl pipette and is left there for 5 min. The chip is lifted and rinsed for 20 s with deionized water. The chip is placed on a paper towel so that most of the water is removed.

2.5. Microfluidic platform

A microfluidic platform (see Fig. 3 right) was fabricated to deliver the sample solutions to the biosensor. It consists of two $45 \times 20 \times 1.6\,mm^3$ PMMA plates, a $10 \times 10 \times 0.15\,mm^3$ PDMS gasket, 1 cm of large tubing (Saint-Gobin Tygon R-3603 AAC00001), and 25 cm of small tubing (Dow Corning 508-002). The top plate has an inlet and outlet port and a 1 mm wide open channel on the bottom side connecting both ports. The inlet has a notch so that the large tubing can be fitted on it. A milling machine (Sherline 8541) was used to create these features. The inlet and outlet ports were drilled with a 0.45 mm drill bit and the channel and the notch were milled with a 1 mm end mill. One end of the small tubing was fitted on the large tubing, and the other end was fitted on the needle of the syringe before testing. The bottom plate covers the open channel. It was joined to the top plate using a clamp and acetone. The PDMS gasket creates a seal between the outlet of the microfluidic platform and the chip. A PDMS sheet was spin coated (Laurell WS-400-6NPP-LITE). The sheet was cut with a scalpel and a hole was punched through it with a syringe needle. The gasket was aligned over the outlet hole on the microfluidic platform.