MEMS BASED IMPEDANCE BIOSENSOR FOR RAPID DETECTION OF LOW CONCENTRATIONS OF FOODBORNE PATHOGENS

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ABSTRACT

An impedance based MEMS biosensor for detection of *Escherichia coli* O157:H7 in food products was designed, fabricated and tested. The device was especially designed with regions for focusing the *E.coli* cells into the centerline of the microchannel, trapping that surrounds the detection electrode to confine and facilitate the contact and binding of *E.coli* antigens with *E.coli* antibody immobilized on the detection electrodes. The devices were tested with and without applying AC signal on the focusing electrodes. The results demonstrate the addition of the focusing capability has increased the strength of the measured signal by a factor of 9, and high sensitivity of 13 cell/ml and detection time of 30 minutes which is significantly quicker than traditional bacterial culture, PCR and ELISA based detection.

INTRODUCTIN

Foodborne bacterial pathogens such as *Salmonella*, *Campylobacter*, *Listeria*, and *Escherichia coli O157:H7* cause millions of infections annually in the U.S. The Centers for Disease Control and Prevention estimates that foodborne diseases cause illness in an estimated 48 million people every year, resulting in 128,000 hospitalizations and 3,000 fatalities [1]. In only the last six months of 2012, there were sixteen food related outbreaks in the U.S. These outbreaks were caused by the transmission of pathogens to humans via contaminated fruit, vegetables, meat, drinking water, milk, poultry, and eggs [2]. From an economic perspective, foodborne pathogens have the potential to cause enormous worldwide financial burden due to medical costs and product recalls, such as the ones occurred in August 2012 when a *salmonella* contamination resulted in the recall of mangoes and cantaloupe [3]. It is noted that the aggregated cost due to food recall and foodborne illness in the U.S. is estimated at $77 billion annually [4].

E.coli are Gram-negative rod-shaped bacterium. Most strains of *E.coli* are harmless, some can cause diarrhea, urinary tract infections, respiratory illness, pneumonia, and other illnesses [5]. *E.coli O157:H7* is obviously one of the most dangerous foodborne pathogenic bacteria. An estimation of 73,000 cases of infection and 61 human deaths in the United States were caused by this bacterium each year (Centers for Disease Control and Prevention, 2006). This bacterium is the main reason of hemolytic uremic syndrome and hemorrhagic colitis [6].

The traditional bacterial culture testing method, that are considered the gold standard, are time consuming (2-5 days) to confirm a diagnosis, and laborious [7]. The PCR is faster but still requires 24 hours and overnight shipping [8]. It may also result in false positive. In the last several years, various biosensors for detection of foodborne pathogens have been developed. These biosensors are based on electromechanical [9], electrical [10], magnetic nano particle [11], electrospun [12], surface plasmon resonance [13], and optical detection. With the advances in micro/nanofabrication processes and in the combination process with biological recognition element techniques, several electrochemical biosensors have been fabricated for the detection of biological cells using the impedimetric biosensor.

This paper reports the design and fabrication of a MEMS based impedance biosensor capable of rapid detection of *E.coli* O157:H7 with a low concentration of 13 cell/ml. The device uses a focusing electrode and three sets of interdigitated microelectrode array (IDMA) for detection of *E.coli* independently. The focusing electrode uses p-DEP to focus and concentrate the bacteria cells into the center of the microchannel, while the other three sets are used to detect the *E.coli* bacteria cells by immobilizing specific antibody on the surface of the microelectrode for impedimetric detection. The change in measured impedance due to antigen–antibody binding is detected using an impedance analyzer. We use the antibodies as the bio-recognition elements due to their sensitivity and selectivity to target cells. The detection processes do not require enrichment steps. We also performed comparative studies between the focusing and non-focusing techniques to understand the significance of both cases and their effect on the bacteria detection mechanism.

MATERIALS AND METHODS

Biosensor Design

The biosensor as shown in Figure 1 consists of (1) A region for focusing the bacteria using a ramp down vertical electrode pair made of electroplated gold along with tilted thin film finger pairs (45^o) with a ramp down channel that generates p-DEP forces to focus and concentrate the bacteria into the center of the microchannel, and direct them toward the sensing microchannel. This technique resulted in highly concentrated samples. (2) A region for cell trapping that surrounds the detection electrode and uses a series of vertical electrode pairs with elliptical shape that generate n-DEP forces pushing the cells toward the region of low E-field gradient on top of the

detection electrodes. Thus, they confine and facilitate the contact and binding of *E.coli* O157:H7 antigens with the *E.coli* O157:H7 antibody. (3) A region for pathogens sensing consists of a series of 3 sets of interdigitated microelectrode arrays (IDMA) with number of fingers equals to 10 pairs made of thin gold film. The finger width, and spacing between fingers are 10 μm, and 10 μm, respectively. A pair of bonding pads stretches out in both directions to be used for electrical connections.

The focusing microchannel width starts at 300 µm and ramp down to 100 µm at the end the microchannel. The focusing microchannel is then divided into three 33 µm wide channels. The outer two channels continue towards the waste outlets, while the center channel is designed to

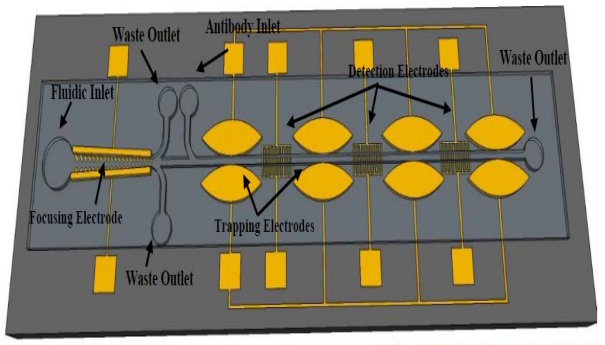

Figure 1: 3D schematic of the impedance biosensor showing the electrode arrays and the microchannel.

carry the bacteria towards the detection region. The depth of the microchannel is 25 µm throughout its length and has a total of four inlet-outlet fluidic ports.

Biosensor Fabrication

The impedance biosensor was fabricated on top of a glass substrate using a series of surface micromachining, photolithography processes. The cross-sectional view of biosensor is shown in Figure 2. The device was fabricated as follow: (1) Initially, the substrate was coated with a thin layer of SU-8, soft baked and exposed to UV light, and hard baked at 150 C for 30 minutes. (2) Chromium (Cr) and gold (Au) thin films were sputter-deposited and patterned to form the detection electrodes, traces and bonding pads, and seed layer at locations corresponding to the ramp down focusing electrodes and trapping electrodes. An optical image of the focusing electrodes is shown in Figure 3. (3) AZ4620 photoresist was patterned to form a mold for electroplating gold for the focusing and trapping electrodes. The electrode height was 25 µm. (4) The photoresist was removed using acetone, and the Cr layer was etched. (5) The microchannel with a thickness of 25 µm was formed using SU-8.

Finally, a thick Polydimethylsiloxane (PDMS) layer was prepared with fluidic connectors for the inlets and outlets in order to seal the microchannel. The PDMS was

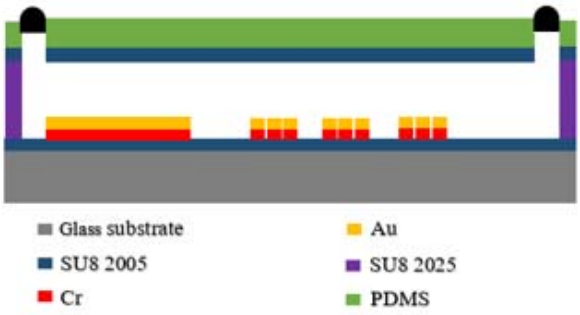

Figure 2: A cross-sectional view demonstrating the layers of the impedance biosensor. The focusing vertical wall and the trapping electrodes are not shown.

treated with oxygen plasma, aligned, and then bonded to the SU-8 microchannel and baked on a hotplate at 65∘C for 10 minutes by placing a heavy object on top of it. This ensures secure bonding between the PDMS and the device substrate. The fabricated Impedance biosensor with (PDMS) is shown in Figure 4.

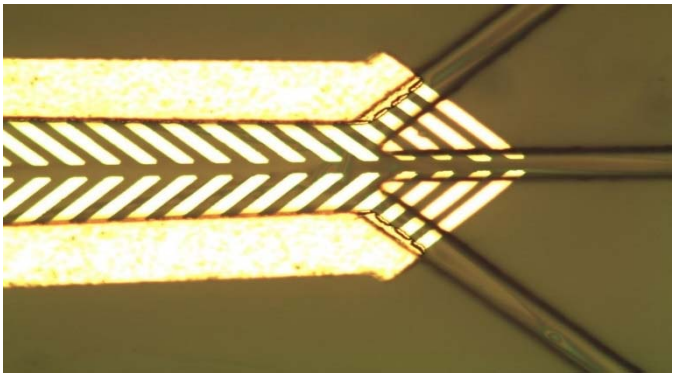

Figure 3: An optical image of the focusing electrode and the SU8 channel branches.

Antibody Immobilization

Goat anti-*E.coli O157:H7* polyclonal antibody (Ab) was purchased from Meridian life sciences, USA. The antibody was prepared at the concentration of 10 µg ml-1 using sterile DI water. 1 ml of prepared antibody was pumped into the microchannel through the inlet and the pump was stopped before the solution reached the outlet. The antibody solution was kept in the microchannel for 1 h, to achieve highest surface coverage, and minimizing any subsequent nonspecific adsorption. The anti-*E.coli O157:H7* antibody passively immobilized on the sensing electrode surface. After 1 h; the liquid was then pumped out, and any unbound antibodies were washed away using sterile DI water.

Antigen Capturing

The *E.coli O157:H7* cells were pumped into the microchannel through the inlet and the flow was stopped before reaching the outlet for 30 minutes. This time duration allowed the *E.coli*

cells to bind with the *E.coli* antibody. After this process, the unbound bacteria washed out using sterile DI water. The immunocomplex (antigen-antibody binding) on the surface of the electrode analyzed for changes in measured impedance. Where the impedance value is directly propositional to the number of bacteria captured on the sensor. Sterile DI water without bacteria used as the control for the experiment [14-18].

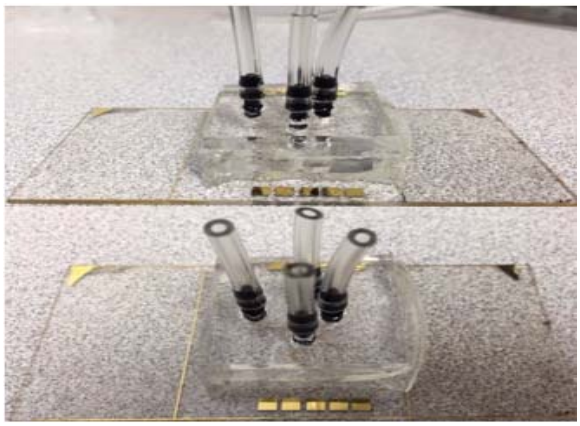

Figure 4: completed fabricated device.

RESULTS AND DISSCUSION

Focusing Effect

To demonstrate the focusing effect, polystyrene microbeads that have similar electrical properties to the cells were used to test the working principle of the biosensor under the focusing effect. Practically, the amplitude and the frequency of the applied signal were adjusted to generate positive dielectrophoresis (p-DEP). Microbeads with diameter of 6 μm with DI water were injected from the inlet of the device and hence the microbeads were focused in the center of the microchannel when an AC E-field (4.5 V peak-to-peak at 5 MHz) was applied across the focusing electrodes. Figure 5 shows the effect of the focusing electrode on the microbeads under the AC influence. Similar approach was demonstrated on the biological cells, a very small flow rate 1-2μL/ minute was used to roll the cells into the center of the microchannel toward the detection region.

Impedance Response without Focusing Effect

Two different concentrations of *E.coli O157:H7* (1000 cell/ml, and 13 cell/ml) were prepared and tested without using the focusing electrodes. The number of cells in each sample were experimentally determined via cell culturing. Initially, the impedance of the interdigitated electrode array was measured using Agilent 4294A impedance analyzer over a frequency range of 100 Hz–10 MHz. These measurements were taken as a baseline. Then, *E.coli* bacteria cells were delivered to the microchannel through the sample inlet. The impedance of the interdigitated electrode array was increased. This demonstrates that the bacteria cells were successfully bound to the adsorbed antibody on the electrode surface. Measurements were recorded also after injecting the bacteria cells. By subtracting the baseline impedance (antibody impedance) from the (antigen-antibody) binding impedance, we obtain the difference which is corresponded to the concentration amount of the bacteria cells. Each experiment was repeated 6 times and the results were obtained and recorded.

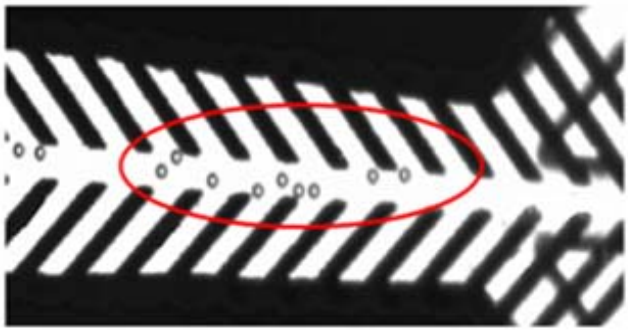

Figure 5: The device was tested using polystyrene microbeads under DEP focusing effect, the microbeads are moving in the center of the channel.

Impedance Response with Focusing Effect

The sensitivity of the biosensor device through the interdigitated microelectrode to detect *E.coli* bacteria samples was studied using impedance spectroscopy. DEP focusing effect was demonstrated beside the sensitivity. Experimentally, function generator was used to apply voltage and frequency to the focusing region of the biosensor device. The amplitude and the frequency were determined and adjusted to certain values that would generate positive dielectrophoresis (p-DEP) effect. The detection IDE array results for the same concentrations showed that the measured impedance was directly proportional to the concentration of bacteria that is bound to the antibody on the detection surface and it was significantly higher. For instance, the concentration of 13 CFU/ml showed (9 times) higher than the impedance value obtained without the focusing effect. The impedance values increased notably. Therefore, improved the quality of capturing the *E.coli* cells on the detection IDE array, and hence improve the measurement sensitivity. Figure 6 shows the combination of focusing and non-focusing effect for 13 and 1000 cell/ml.

Specificity Testing

To confirm the specificity of the biosensor device, the anti *E-coli* antibody was immobilized on the detection electrode and was tested with different antigen (*salmonella typhimurium*). The measured response showed no difference in the impedance measurement values with respect the baseline (antibody) response of the IDE array. This was predicted since the detection electrodes were modified specially using *anti-E.coli O157:H7* antibody. The impedance response is shown in Figure 7.

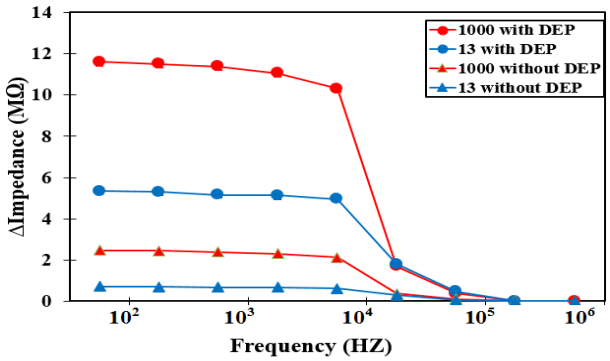

Figure 6: The impedance response of the biosensor device with and without DEP focusing effect for concentrations of 13 cell/ml and 1000 cell/ml.

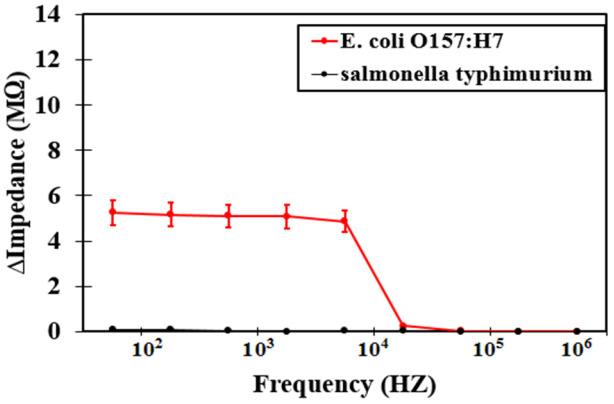

Figure 7: The response of salmonella typhimurium and E.coli O157:H7 to an anti-E.coli O157:H7 antibody.

CONCLUSION

The work presented in this paper involves a MEMS biosensor based on impedance spectroscopy for the detection of *E.coli O157:H7* bacteria cells. The device consists of interdigitated microelectrode arrays and microchannel. The device was tested over the frequency range of 100 Hz to 10 MHz frequency and the results demonstrate that the p-DEP increases the response sensitivity by a factor of (9 times) depending on the bacterial concentration. The device was able to detect *E.coli* O157:H7 concentration as low as 13 CFU/ml.

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