

DETECTION OF BACTERIAL CELLS BASED ON MICRO-CHANNEL GATING

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ABSTRACT

In this communication, we describe a novel methodology for single bacterial detection based on micro-channel gating and involving the use of microelectrodes in a micro-channel for detection of target cell capture. The sensors designed have been shown to be capable of electrical detection of the target cells at the single cell level.

Keywords: Bacterial Detection, Electrical Detection, Label-free detection

1. INTRODUCTION

Current microbiological techniques used for detection of pathogens involve expensive and time consuming methods such as culture enrichment and plating techniques, which can take several days [1]. Described below is a rapid and inexpensive technique which can be used to detect single bacterial cells electrically (label-free format) in real-time. We have successfully demonstrated real-time detection of target cells by measuring instantaneous changes in ionic impedance.

2. THEORY

The cross section of the biosensor used in this study is presented in Fig. 1. Electrodes are labeled A, B, and C for reference. A third gold electrode, B, is included in the active area of the channel, allowing for immobilization of antibodies with an affinity to bind to target bacterial cells in the active area of the sensor. Gold electrodes are very suitable for surface chemistry modifications, like deposition of surface assembled monolayers, which can optimize antibody immobilization. A sample suspicious of bacterial cell contamination is injected into the micro-channel. If the sample contains the targeted bacteria, the cells will attach to the electrodes, partially clogging the channel thus resulting in ionic resistance increase. By monitoring the impedance across micro-electrodes A and C, it is possible to detect the channel gating caused by bacterial cell attachment inside the channel. By choosing channel and electrode geometries close to the cell size, the sensitivity of the device can be increased down to single cell detection.

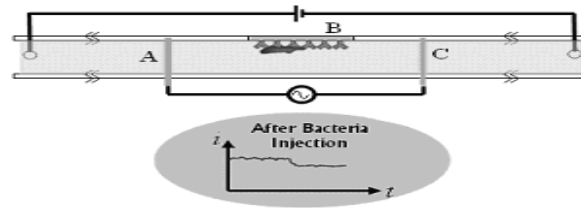


Figure1. Cross Section schematic of gated micro-channel with electrodes labeled A, B, and C. The targeted bacteria bind to the antibodies which are immobilized on the gold electrode. (Bottom plot) Prediction of current after injection of bacteria.

3. EXPERIMENTAL

Presented in Fig. 2a is a schematic design of the micro-fluidic biochip used, consisting of a set of microelectrodes on a glass substrate and a channel right above, embedded in PDMS (fabricated by the Stanford Microfluidics Foundry). The electrodes were constructed onto glass using sputtering and then lift-off (Fig. 2b).

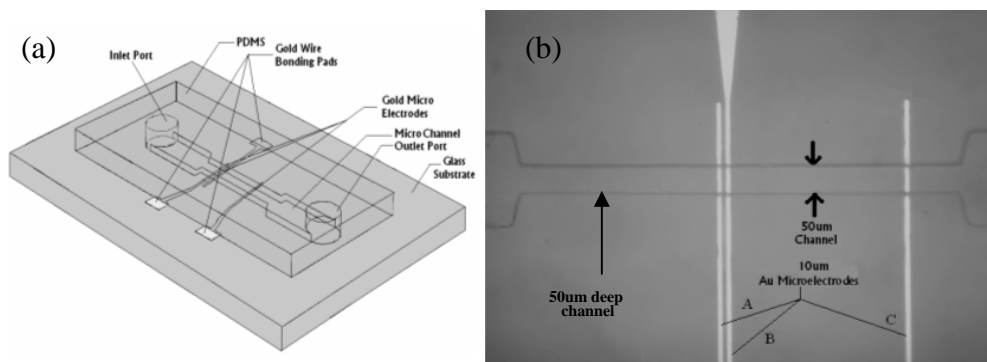


Figure 2. (a) Schematic of microfluidic chip used in this study. (b) Optical micrograph of top view of fabricated device with electrodes labeled A, B, and C. Electrode B was not used in this study.

In this study, yeast cells were used instead of bacterial cells, due to their similarity in size and dielectric properties, and Concanavalin A (ConA), a glycoprotein with affinity for yeast, in place of antibodies in order to obtain preliminary results and demonstrate the success of the technology. Immobilization of ConA on the electrodes was carried out by physical adsorption. ConA solution was injected and incubated into the channel for 15 minutes, then activated by the injection of Mn^{2+} , Mg^{2+} , and Ca^{2+} ions. A 200mM KCl solution in 10mM Hepes buffer with a pH of 6.8 containing yeast was injected into the channel at a flow rate of 100nl/min. The electrical impedance was measured over time between electrodes A and C at a frequency of 29.8 kHz. At this frequency the solution resistance dominates the impedance. The channels were also monitored using optical microscopy simultaneously as electrical impedance was measured.

4. RESULTS AND DISCUSSION

Experiments were performed on various channel sizes. Figure 3a shows yeast cells being captured by the receptors on the electrode surface in the 50um deep channel. Results in figure 3b show an instantaneous increase in electrical impedance as a small number of cells bind to the surface of the electrodes, demonstrating real time detection of cell capture.

Experiments were also performed with a 10um deep channel. In this particular experiment, no receptors were immobilized onto the electrodes, so all capture was a result of non-specific binding. Figure 4a and 4b shows cells being captured on the electrodes and clogging the channels. As shown in figure 4c, the impedance ramps up at a relatively steady rate as result of cell accumulation and drops instantaneously as a result of cells unbinding from the surface and the channel being cleared up. The unbinding of cells results from an increase in the fluid pressure.

For channel sizes comparable to the diameter of yeast (5um), nonspecific binding and channel clogging have been shown to be problematic. Larger channels are more practical for implementation, since they are sensitive enough to electrically detect the presence of a

small number of cells, while at the same time minimizing channel clogging and nonspecific binding.

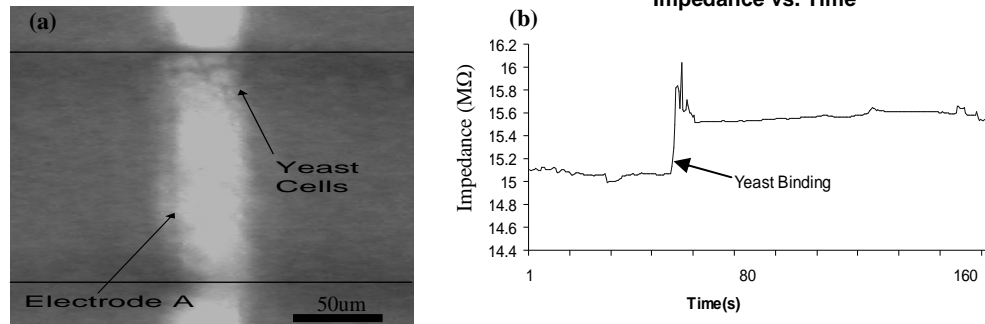


Figure 3. (a) Optical micrograph of gold electrode A after yeast binding has occurred. Electrodes B and C not shown. (b) Impedance vs. time. Impedance jump at $t = 55$ s due to yeast binding.

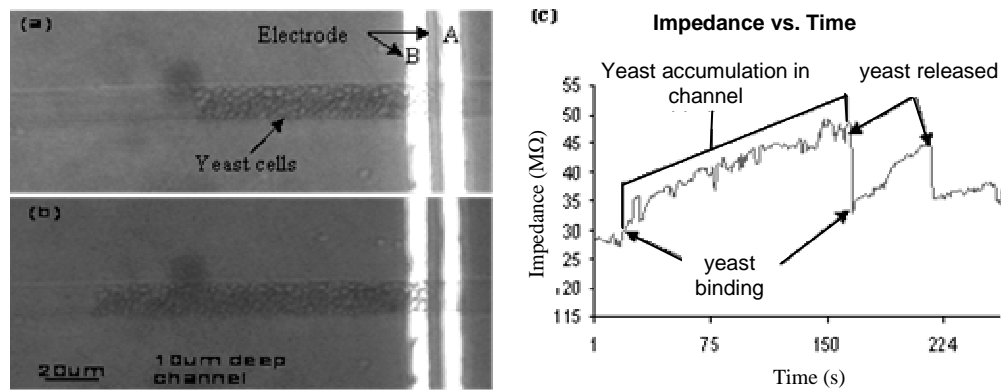


Figure 4. (a) Optical micrograph of yeast cells accumulating in channel at $t = 75$ seconds. (b) at $t = 130$ s. Electrode C is to the left and is not shown in this figure. (c) Plot of impedance over time. Impedance increases steadily as cell's accumulate in channel. Release of cells results in impedance drop at $t = 160$ s. The same cycle is repeated until $t = 220$. No cells across electrodes after $t = 220$ s.

5. CONCLUSION

A label-free electrical detection platform for bacterial cells based on microchannel gating was developed. We have shown that this technology is capable of electrically detecting the presence of a very small number of target cells. We believe that, with proper optimization and modification, this type of sensor can be used to detect many different types of pathogens and potentially even cancer cells in blood.

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REFERENCES

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