BioMEMS and Bionanotechnology and Applications to Biological Sensing

Rashid Bashir, Liju Yang, Demir Akin
Birck Nanotechnology Center and Bindley Bioscience Center, School of Electrical and Computer Engineering, Weldon School of Biomedical Purdue University, West Lafayette, IN 47907

ABSTRACT

Biomedical or Biological Micro-Electro-Mechanical-Systems (BioMEMS) and Bionanotechnology has found widespread use in a wide variety of applications in diagnostics, sensing, and characterization of biological entities. This paper reviews some of the interdisciplinary work performed in our group in the recent years to develop micro-integrated devices to characterize biological entities. We have used electrical based phenomena to perform characterization and various functions needed for integrated biochips. One sub-system takes advantage of the dielectrophoretic effect to sort and concentrate bacterial cells and viruses within a micro-fluidic biochip. Another sub-system measures impedance changes produced by the metabolic activity of bacterial cells to determine their viability. The last sub-system described has been used to detect the charge on DNA molecules as it translocates through nanopore channels. These devices with an electronic or mechanical signal output can be very useful in producing practical systems for rapid detection and characterization of cells for a wide variety of applications in the food safety and health diagnostics industries.

INTRODUCTION

BioMEMS and microfluidics are at present a heavily researched area with a wide variety of important biomedical applications[1]. The ability to fabricate micro and nano-structures with scales and dimensions similar to biological entities has paved the way to new concepts and systems for a variety of cellular, diagnostic and therapeutic applications, such as intelligent biochips and biosensors[2]. In recent years, we have devoted our efforts to develop bioMEMs devices and demonstrate their applications in sorting and concentrating bacterial cells using dielectrophoresis technique, measuring the metabolic activity of bacterial cells using impedance measurement, and detecting DNA molecules in nanopore channels by electrical signal.

DIELECTROPHORESIS AND ANTIBODY MEDIATED SELECTIVE CAPTURE OF *LISTERIA* CELLS ON A MICRO-FLUIDIC CHIP

The technique of dielectrophoresis (DEP) and manipulation of biological particles by electrical forces provide unique means to control the separation dynamics of biological agents as most biological cells or bacteria behave as dielectric particles in external electric field[3]. We have demonstrated that dielectrophoresis is capable of separating live and heat-killed *Listeria* bacteria on microfabricated interdigitated electrodes in a static solution[4], as well as different biological particles in a micro-fluidic device in the dynamic flow by considering the differences in their sizes and dielectric properties[5]. However, in many cases, the dielectric properties and the sizes of the different particle types are not significantly different, as a result, the differential driving force acting to the particles will not be sufficient to separate different types of particle. The challenge therefore exists to develop micro-systems which are capable of separating biological cells with similar size and dielectric properties. We proposed to combine the advantages of DEP concentration and antibody selective capture, which demonstrated selective capture of target cells from a mixture of cells with similar dielectric properties in a micro-fluidic biochip.

The micro-fluidic biochip used in the DEP mediated selective capture was fabricated as our previous report[5]. Monoclonal anti-*Listeria* antibody C11E9 was immobilized onto the surface of DEP chamber through biotin-streptavidin chemistry. *Listeria monocytogenes* V7 cells and *Escherichia coli* K12 cells were used to demonstrate the function of the biochip. Fig. 1 shows the images of *L.*

monoctygenes and *E. coli* cells captured in the micro-biochip by the DEP and antibody selective capture. It can be seen that there are some *L. monocytogenes* cells left on the surface of the channel after DEP was turned off and washing with DI water, while *E. coli* bacteria are almost all gone. Without DEP, no *L. monocytogenes* cells could be seen on the channel surface (picture not shown). The antibody capture efficiency was between 20%-30% for cell numbers in the range on 10^1 to 10^4 cfu/5µl. Compared to the capture efficiencies of antibodies immobilized on a solid surface to bacterial cells of 0.5% by placing a droplet (containing ~10^5 cells) directly on the electrode surface and 0.01% by immersing the electrode into 1 ml solution containing 10^7 cells[6]. In addition, this techniques can be used to concentrate bacteria, cells, viruses, DNA, or proteins, as long as their dielectric constant is different than the dielectric constant of the medium that they are suspended in. This technique can be used to separate virus particles from other particles in air, or used to concentrate particles of a particular type in a micro-chamber of interest[7].

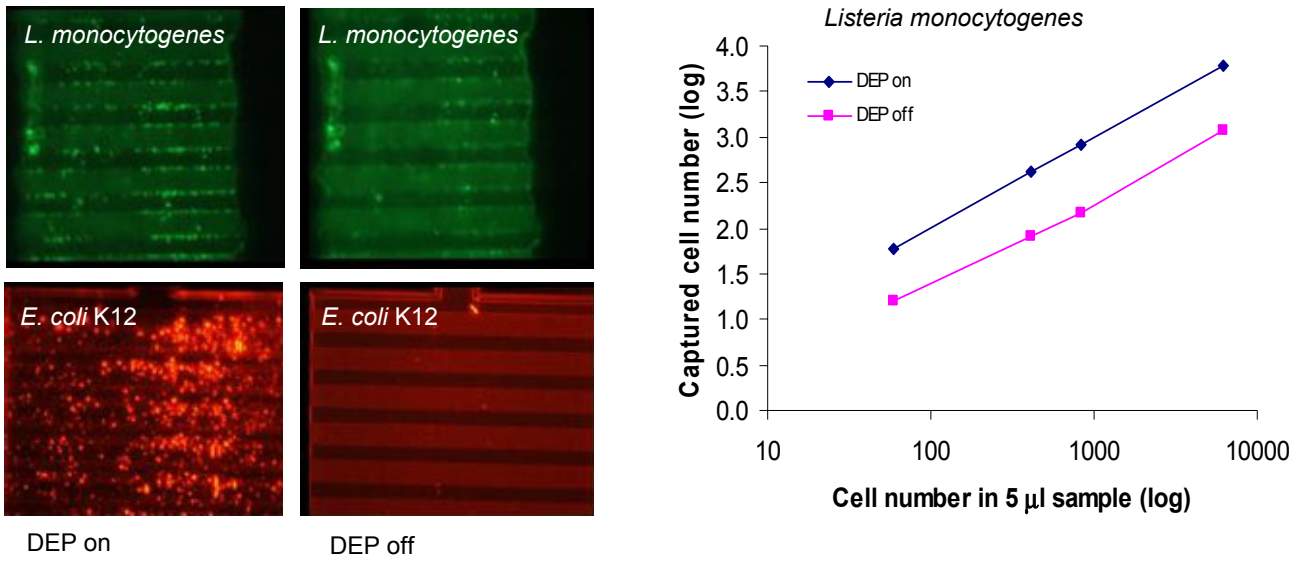

Fig. 1 Images of captured bacterial cells when DEP was on and off in microchip immobilized with anti-*Listeria* antibodies and cell number change upon turning off DEP for samples containing different cell numbers.

MICRO-SCALE BACTERIAL CELL CULTURE IN A CHIP

The metabolic activity of viable microorganisms changes the ionic concentration of the medium in which they are suspended, by the secretion of ionic metabolic byproducts. This change can be detected by measuring the AC impedance of metallic electrodes in contact with the medium[8]. Large scale systems for impedance-based detection of bacterial metabolism have been in use for a long time. Unfortunately, the detection time of the conventional impedance-based methods is quite long when the concentration of bacterial cells present in the sample is very low. This limitation can be overcome if those few cells are concentrated into a very small volume while the impedance is being measured. We have designed a micro-chip in which a few bacterial cells can be concentrated from a dilute sample into volumes on the order of nanoliters[9]. Such application of a microfabricated device can completely eliminate the requirement for amplifying the bacterial population by enrichment in many conventional methods, thus the sensitivity and speed of the assay can be greatly improved.

In recent work, we have fabricated devices consisting of a network of channels and chambers etched in a crystalline silicon substrate[9]. The biochip consists of a large straight channel (main channel) containing DEP deviation electrodes and a 400 pl incubation chamber containing DEP concentration electrodes and impedance measurement electrodes. Through the main channel the sample can be flowed at the desired rate while the cells in it are deviated by DEP forces into a small channel that leads into the

concentration/incubation/detection chamber. Cells were concentrated in the incubation chamber when DEP concentration electrodes were active, and metabolic activity of the concentrated cells was then monitored by impedance measurement. With the optimal combination of medium conductivity, the flow velocity, and DEP excitation voltage and frequency, 100% concentration efficiency can be expected. We have shown that the sterile media did not exhibit any clear metabolic signal. The bacterial sample injected without the DEP concentration generated a metabolic signal corresponding to the exponential growth at approximately 7.5 h incubation time, because the number of cells stayed in the incubation chamber was very low, whereas the metabolic signal can be observed in the first hour for both samples with DEP concentration. It was clearly demonstrated that the DEP-driven concentration reduced dramatically the length of the detection time for the metabolic signal, and this would be especially useful for very dilute bacterial suspensions. By taking the advantage of DEP concentration, collecting a large percentage of the cells from a very dilute sample (40 µl) in the 400 pl detection chamber, the deviation and concentration system makes the effective cell concentration in the chamber with the concentration factor between 10^4 and 10^5 of magnitude higher than that in the original sample, assuming that between 10% and 100% of cells were captured. These measurements indicated that the metabolic activity of a minimum of 50 to 200 cells can be detected. The device is currently being redesigned to increase its sensitivity to analyze a single cell.

DNA CHARGE DETECTION BASED ON IONIC CURRENT FLUCTUATIONS USING NANOPORE CHANNELS

Nanoscale pores within biological or artificial membranes acting as mechanical gating elements are very promising devices as single molecule sensors for the rapid characterization and sequencing of nucleic acid molecules. Our group has pursued the study of single DNA molecule sensors using the nanopore. The nano-pore channel (Fig. 2 (a), (b)) with a diameter of ~6 nm and the length of 50-60 nm was fabricated and assembled as our previously report[10]. Purified 200bp DNA fragment from the human CRISP-3 gene with a final concentration of 0.3 µg/ml was applied to one chamber and the current measurements from that chamber to the other chamber were made.

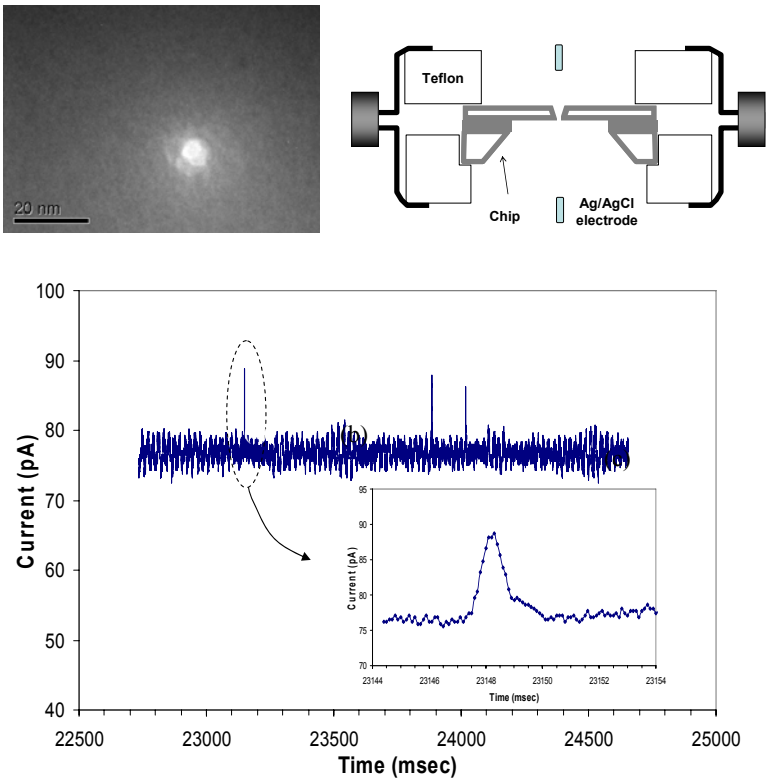

Fig. 2 (a) The transmission electron microscope (TEM) image of the smallest diameter of the nanopore channel in the oxidized silicon membrane. (b) Cross-section of the oxidized silicon membrane. (c) Typical pulses measured for the translocation of the 200bp dsDNA through the nanopore channel. The inset shows one pulse in more detail (ref. 10).

Fig. 2 (c) shows the typical pulses measured for the translocation of the 200bp dsDNA through the nanopore. When the 200bp dsDNA was applied to side 1 and as the dsDNA passed through the pore, it modified the bulk and the interface currents resulting in typical current pulses. Since the DNA molecule is around the same length as the channel, the current is expected to increase and then decrease as the DNA leaves the pore, as measured.

The experimental results presented here demonstrate that the charge on the DNA can be detected in the nanopore channel due to an inherent charge amplification and transduction of the DNA charge into ionic current in the mobile surface charge, similar to a field effect transistor. The demonstration of electrical gating effect would allow one to use these nanopore membranes as the third electrode capable of characterizing multiple threads of dsDNA simultaneously. The electrical signal from this device for the passage of short dsDNA molecule with mismatches could exhibit a difference when compared to the signal from matched strands providing a label-free means to detect DNA hybridization.

CONCLUSIONS AND FUTURE DIRECTIONS

We have demonstrated three bioMEMs sub-systems and devices that have been designed for pre-test cell concentration or sorting, integration of cell concentration and sequential detection of metabolic activity, as well as the ability to analyze the constituents of a cell such as DNA in a micro-chip. The results have shown that bioMEMS devices are ideally suited for studies of handling, manipulation, and characterization of cells and microorganisms. The goal of such an effort should be directed towards a completely microsystem that can handle, detect, and characterize a single cell or microorganism. As shown in Fig. 3, such a system should be able to perform all functions needed for rapid detection such as cell sorting, cell concentration, and cell detection. Cells can be sorted and captured using antibodies, cultured to investigate their metabolic activity, and then lysed to characterize and detect specific molecules from individual cells using nano-sensors such as nanowires, cantilevers, and nanopores. Such endeavors can not only yield very important scientific results but can also be used immediately in practical diagnostic applications in the health and food industry, and in biological and chemical hazard prevention systems.

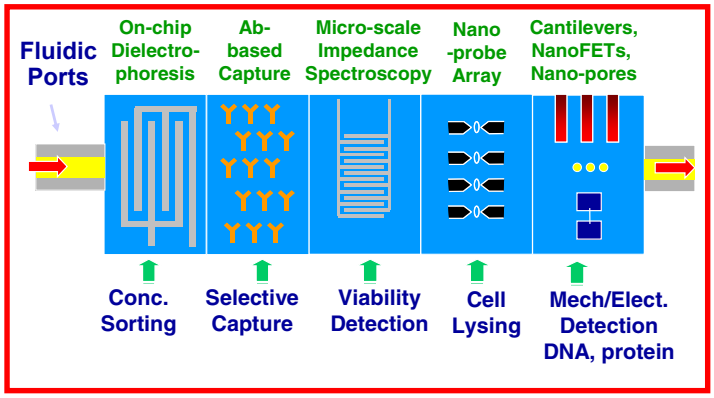

Fig. 3: The functions needed in an integrated biochip for rapid cell detection

ACKNOWLDGEMENT

The authors would like to acknowledge the support of the Birck Nanotechnology Center and the staff of the Purdue University Microfabrication facilities for wafer processing. We would also like to acknowledge the support of the NSF Career Award, Center for Food Safety Engineering at Purdue University through a USDA cooperative agreement: CRIS 1935-42000-035-00D, agreement # 58-1935-9-010, NASA Institute of Nanoelectronics and Computing (INAC) at Purdue University under award no. NCC 2-1363. The authors would also like to thank Prof. M. Ladisch, Prof. Arun Bhunia of Purdue University and Dr. Farhad Kosari and Prof. George Vasmatzis of Mayo Clinic, for their collaboration and many valuable discussions.

REFERENCES

1. M. Ferrari (Ed.), *Biomedical Nanotechnology, Vol. I-IV*, Kluwer Academic Publishers, 2005, in press.
2. R. Bashir, "BioMEMS: state-of-the-art in detection, opportunities and prospects", *Advanced Drug Delivery Reviews"*, **56**, pp. 1565-1586, 2004.

3. R. Pethig, and G.H. Markx, “Applications of dielectrophoresis in biotechnology”, *TIBTECH*, **15**, 426-432 (1997).
4. H. Li, and R. Bashir, “Dielectrophoretic separation and manipulation of live and heat-treated cells of *Listeria* on microfabricated devices with interdigitated electrodes”, *Sens. Actuat. B*, **86**, pp. 215-221, 2002.
5. H. Li, Y. Zheng, D. Akin, and R. Bashir, “Characterization and Modeling of a Micro-Fluidic Dielectrophoresis filter for Biological Species”, *J. Microelectromechan. Sys.,* **14**, 103-112 (2005).
6. J. D. Brewster, A. G. Gehring, R. S. Mazenko, L. J. Van Houten, and C. J. Crawford, “Immunoelectrochemical Assays for Bacteria: Use the Epifluorescence Microscopy and Rapid-scan Electrochemical Techniques in Development of an Assay for *Salmonella*”, *Anal. Chem*. **68**, 4153-4159 (1996).
7. D. Akin, H. Li, and R. Bashir, “Real-time virus trapping and fluorescent imaging in microfluidic devices”, *Nano Letters*, **4**, pp. 257-259, 2004.
8. R. Eden and G. Eden, *Impedance Microbiology*, Research Studies Press Ltd., 1984.
9. R. Gómez, D. T. Morisette, and R. Bashir, “Impedance microbiology-on-a-chip: Microfluidic bioprocessor for rapid detection of bacterial metabolism” *J. Microelectromechan. Sys.* **14**, 829-838, 2005.
10. H. Chang, F. Kosari, G. Andreadakis, M. A. Alam, G. Vasmatzis, and R. Bashir, ”DNA-Mediated fluctuations in ionic currents through silicon oxide nanopore channels”, *NanoLetters*, **4**, 1551-1556, 2004.