Single Cell Array Impedance Analysis for Cell Detection and Classification in a Microfluidic Device

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Abstract: Impedance analysis of single cells is presented in this paper. Following the separation of a target cell type by dielectrophoresis in our previous work, this paper focuses on capturing the cells as a single array and performing impedance analysis to point out the signature difference between each cell type. Lab-on-a-chip devices having a titanium interdigitated electrode layer on a glass substrate and a PDMS microchannel are fabricated to capture each cell in a single form and perform impedance analysis. MDA-MB-231 and HeLa cells are used in our experiments.

1 INTRODUCTION

Microfluidic based cell separation and capturing systems with high efficiency, fast response time, multi-functionality, high accuracy and repeatability rates are emerged as a new diagnosis tool for many applications in biotechnology, drug discovery, medicine, chemistry and environmental problems (Mateo et al., 2014, Karabacak et al., 2014). They require minimal sample size, low cost to fabricate, portable and most importantly allow early detection of circulating cancer cells and can be used as a point-of-care product compare to macro-scale cell separation and diagnosis systems (Alix-Panabieres et al., 2014, Jin et al., 2014). In literature, active and passive cell separation systems could be found on the basis of differences in cell's geometry, chemical and electrical properties. Passive systems are based on the flow characteristics of the fluid with particles inside the microchannel and separation is accomplished using the geometrical differences of the particles, whereas active systems require outside source, like magnetic field, electrical field, acoustics and optics, to sort/capture the particle inside microchannel. The outside source applied in the active systems must accomplish the task without damaging the viability of cells (Hajba et al., 2014).

Impedance spectroscopy (IS) is an important measurement system in cell biology in the analysis of cellular structure, cell physiology and cell to disease interaction studies (Park et al., 2010). Cellular resistivity measurement without the need for any molecular marker can provide significant information to researchers on the mechanisms of cell functioning, especially in the formation and progression of a disease. For example, membrane specific capacitances of cancer tissue cells are different than that of the normal cell membranes. And it is well known that white blood cells have different capacitance values among themselves, due to the surface geometry of the cell membrane, and cell membranes with specific apoptosis or necrosis condition have different capacitance and conductivity values compare to the ones at the normal mode of operation. In a recent study (Anh-Nguyen et al., 2016) long-term monitoring of MCF-7 breast cancer cell attachment, adhesion, spreading and the response of those cells to anticancer drug Cisplatin is presented within the same platform. These cellular activities and responses of cancer cells to drug treatment are indicated by impedance spectra of target cells. Another study (Dastider et al., 2016) presents a biosensor for detection of low concentration (39 CFU/mL) foodborne pathogen, E.coli, on a microfluidic platform consist of two dielectrophoretic focusing and impedance sensing sequentially. Positive dielectrophoretic force applied to concentrate the bacteria towards to the center of the microchannel and anti-E.coli coated interdigitated electrode arrays detects the flowing bacteria throughout the microchannel.

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The aim of our research is to fabricate a microfluidic based lab-on-a-chip (LOC) device that will consist of two main parts: In the first part, the cells will be separated by dielectrophoresis (DEP) based on their dielectric properties. In the second part, cells are captured as single cell array by hydrodynamic forces and the cell impedance is measured. The fabricated LOC system has a potential to be used for counting blood cells (hemogram), stem cell count, drug resistance detection, cell phenotype etc. These measurements are currently made with restricted use of the flow cytometer, which requires large volume of samples which are fluorescently labelled and then excited by the laser.

The two parts of our design are studied separately to maintain the simplicity of our research and this paper focuses on the capturing of target cells by hydrodynamic forces and carrying out impedance analysis for cell detection, classification and characterization.

2 DESIGN

The idea is inspired from Tan's study (Tan et al., 2007) where target cells are trapped as a single cell array throughout a two-dimensional microfluidic channel. Interdigitated electrode couples are placed under each trap site to detect captured cells via an LCR meter. The width and the height of the microchannel are close to the diameter of target cells to maintain the stream of cells in a linear form. There are two potential paths with different flow resistances for cells to follow: Path 1 is the trap site with a lower flow resistance when it is empty and Path 2 is the fraction of the main channel. When a cell is trapped in Path 1 consecutive cells are directed to Path 2 due to increase in flow resistance. Tan's design consists of 5µm x 5µm narrow necks to create a trap site in Path 1 and due to the fabrication challenges of such structures we present a 3D polydimethylsiloxane (PDMS) microchannel which is shown in Figure 1 with a minimum feature size of 10µm. A narrow neck in Path 1 is created in vertical direction by limiting the channel height to 5µm in this region and the rest of the channel has a thickness of 15µm.

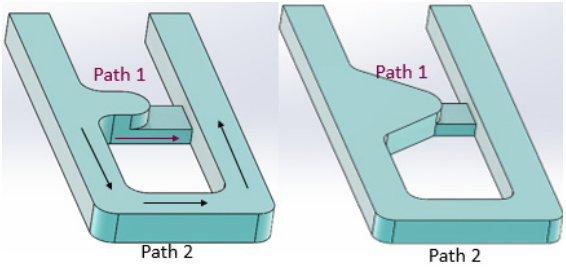

Figure 1: There are two potential paths for cells to follow. Path 1 has a lower flow resistance until a cell is trapped. A triangular trap site has a lower flow resistance than a circular one.

Different geometries for trap site are studied to prevent multiple cell capturing in the same trap site and clogging of main channel. The length and the width of the Path 1 and Path 2 play a key role to determine the flow resistance. Path 1 is designed to be wider and shorter to maintain lowest flow resistance possible. Fluid velocity variation is shown in Figure 2 and it is shown that higher trapping efficiency is achieved by just increasing the channel length due to increase in the flow resistance of Path 2. But multiple cells could be captured at once in the trap area in such structures.

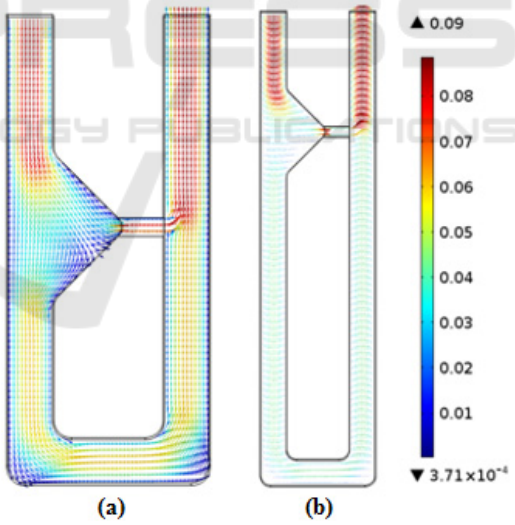

Figure 2: Fluid velocity distributions for different Path 2 lengths. Colour bar: Fluid velocity (m/s).

We present a novel design to maintain capturing single cells only in a graded triangular trap site as seen in Figure 3. When a cell (1) is trapped in the acute angled area the subsequent cells (2) are cleared out of the trap site. This process repeats itself until all the trap sites are filled with single cells.

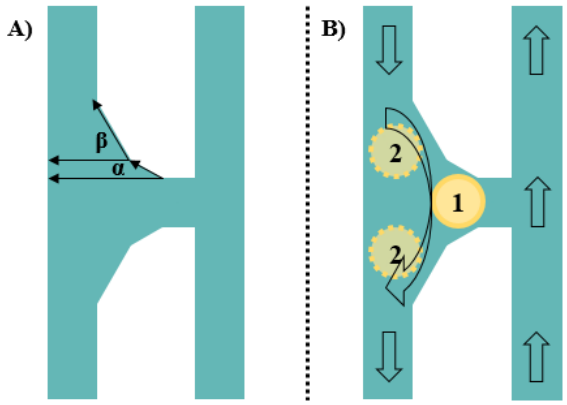

Figure 3: Schematic view of the graded triangular trap design. A) α=30°, β=60° B) When a cell is trapped in narrow region subsequent cells are cleared out of the trap site.

Interdigitated titanium electrode couples are placed under each trap site. The width of the microelectrode fingers is 15µm and the gap between the fingers is 8µm. Schematic view of the whole system is given in Figure 4. There are 40 triangular trap sites in total divided in four parallel lines of 10 trap sites. The signal is recorded for 2 of the 4 parallel lines at the same time by interdigitated electrode array couples connected to an LCR meter.

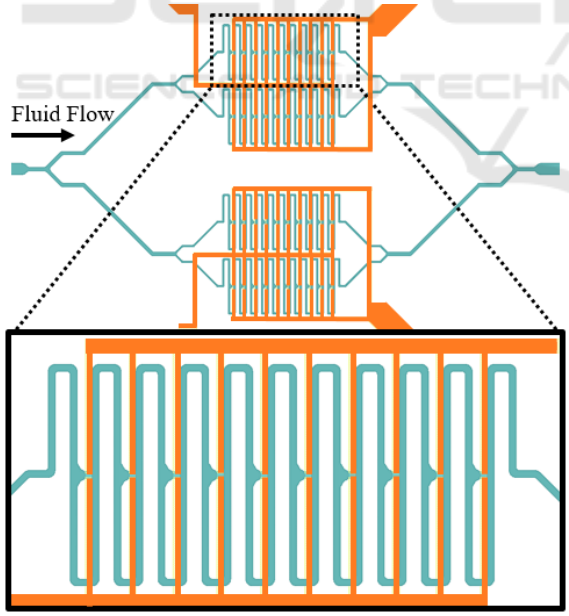

Figure 4: Schematic view of the whole trapping area. There are 40 triangular trap sites in total. *Green lines: microfluidic channel, orange lines: interdigitated microelectrodes.*

3 MATERIALS AND METHODS

Conventional optical lithography processes are used to create 3D microchannel structures by aligning two optical mask to create a master mold for PDMS casting. SU-8 3010 negative photoresist is used for both layers. First layer has a thickness of 5µm and the second layer is 10µm. Path 1 has a continuous opening (transparent) in the first mask only to create a vertical narrow neck in trap sites.

Titanium microelectrodes with a thickness of 200nm are fabricated on glass substrate coated with a 400nm AZ 1505 positive photoresist by DC magnetron sputtering and following lift-off process. PDMS microchannel and microelectrodes coated glass slide are aligned under an optical microscope and plasma activated bonding process applied. A droplet of methanol is used to create a sliding layer between the PDMS microchannel and glass slide during the alignment. Aligned substrates are placed in a vacuum oven at 50˚C for 15 minutes to evaporate the methanol and a stable bonding is achieved.

MDA-MB-231 (human breast cancer cell line) and HeLa cells (cervical cancer cell line) with different medium conductivities are used in our experiments. Conductivity of medium is adjusted by the concentration of PBS (phosphate-buffered saline) in 200mM sucrose solution (1X PBS=15,84 mS/cm, 0.5X PBS=7,71mS/cm).

4 EXPERIMENTS & DISCUSSION

Fluid flow is controlled by a syringe pump to achieve precise flow rates and experiment is observed under an optical microscope. Agilent E4980A Precision LCR Meter (Keysight Technologies, USA) is used for the impedance measurements. Impedance values are recorded for a frequency range of 1-500kHz with an applied potential of $1V_{pp}$.

MDA-MB-231 and HeLa cells are efficiently trapped in triangular sites and the signals are recorded before and after the cells are trapped. Due to the elastic nature of biological cells, some of them are deformed through the bottom neck and slip away. While the hydrodynamic trap design itself is independent from the flow rate, it has been seen that the design is most effective for flow rates below 2µl/min and cells with a minimum diameter of 10µm.

MDA-MB-231 cells are trapped as seen in Figure 5. Yellow circles represent empty traps and green ones for filled traps with a single cell. The impedance shift is recorded with the cells in green circles.

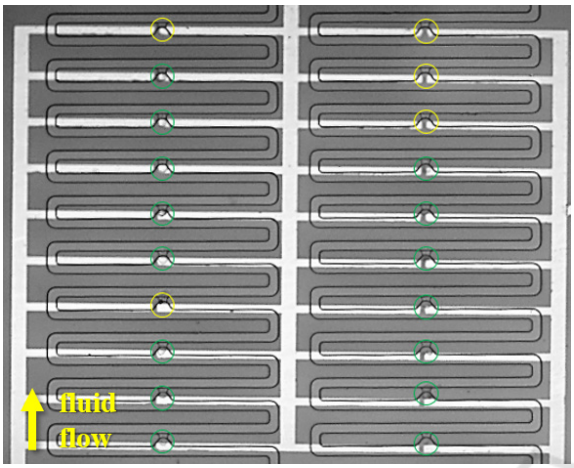

Figure 5: MDA-MB-231 cells in PBS. Graded triangular traps are used. The cells with no contact with microelectrodes or empty traps are shown in yellow circles where the cells are in contact with the microelectrodes are shown in green. Each trap site has a single MDA-MB-231 cell.

A detailed view of filled and empty trap sites is given in Figure 6.

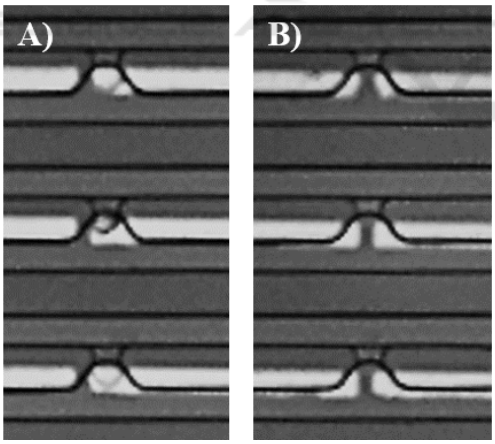

Figure 6: Single cell trap sites: A) filled traps with a single cell B) empty traps.

The impedance readings are recorded continuously before and after releasing the cells into the microchannel. When a cell is trapped the impedance is shifted depending on the cell properties. It can be seen that the impedance shift (ΔZ) varies for each cell line which can be used for further analysis or diagnosis applications. ΔZ values for MDA-MB-231 and HeLa cells in 1XPBS are given in Figure 7, showing larger shifts for MDA-MB-231 cells compare to HeLa cells. This result further proved that the impedance measurements are sensitive to cell types.

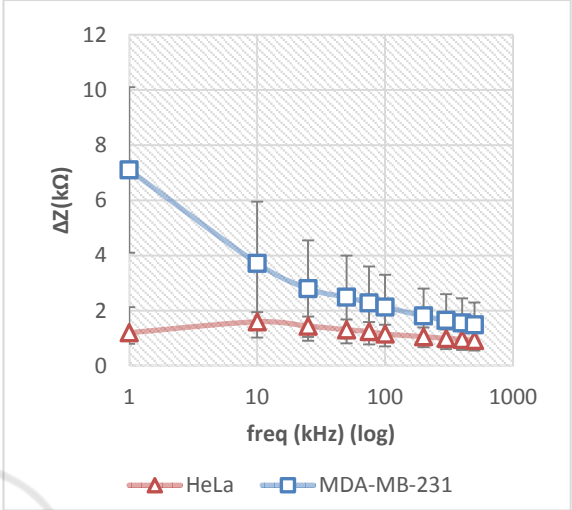

Figure 7: Impedance shift of MDA-MB-231 and HeLa cell lines in 1XPBS medium.

ΔZ values for MDA-MB-231 cells in 1XPBS and 0.5XPBS mediums are given in Figure 8. It is found that the magnitude of ΔZ increases with decreasing conductivity of medium. This result shows that medium conductivity can be adjusted to achieve higher sensitivity for a target cell line.

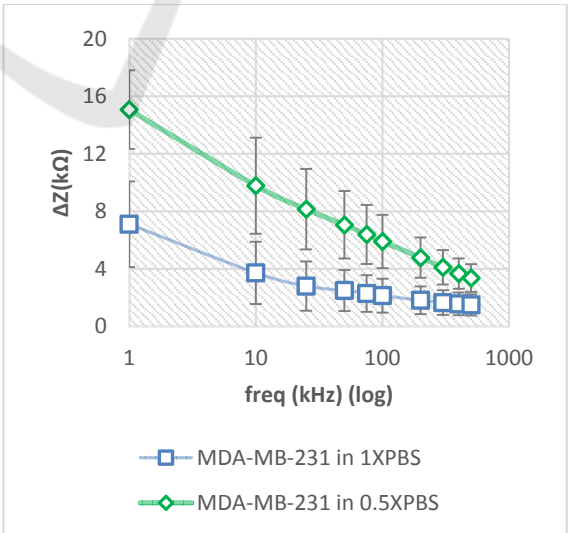

Figure 8: Impedance shift of MDA-MB-231 cell lines in 0.5XPBS and 1XPBS mediums.

The signal is collected from the array of 20 single cells in our current design but it is proven to maintain these target cells in these trap sites throughout the impedance analysis. As a future work of our research, individual signals will be collected from individual interdigitated electrode couples placed under each trap sites to gain the long-term data of impedance analysis of individual cells.

There is a recent study also inspired by Tan's design (Zhou et al., 2016) that presents hydrodynamic trapping and impedance spectroscopy of single cells. It has been shown that with a similar design of our own, it is possible to monitor dynamic changes in electrical properties of individual cells over long periods of time to investigate the external effects on cells.

5 CONCLUSIONS

Our experimental results show that diagnosing of different cell lines in mediums with an optimum conductivity is achievable using current single cell trap array. The impedance shift is sensitive to cell type and it can be used for the estimation of the total number of captured target cells. The cell would be stimulated by different chemicals or drugs injected to microsystem to see the effects on cell viability or its electrical properties. Further studies will focus on introducing the optimum medium conductivity and a frequency value for a target cell line to record the impedance shift with a minimum error. This technique will be used for estimating the physical/electrical properties of cell structures and the separation efficiency by DEP will be increased with gained knowledge of target cell lines.

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