Single cell array impedance analysis 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. HCT116 (homosapiens colon colorectal carcino) and HEK293 (human embryonic kidney) cells are used in our experiments.

1. Introduction

Introducing a lab-on-a-chip (LoC) device with two different active regions to define single cell physical/electrical characteristics for diagnosing a disease and proposing a potential drug is our primary research goal. In the first active region of the LoC, target cells are separated by dielectrophoresis (DEP) and transferred to second active region and the cells are captured as a single array in hydrodynamic traps where the impedance measurement is carried out. The final form of the LoC has two active regions on the same body. For simplicity of our research, those regions are studied distinctly. This paper focuses on the second region and preliminary outcomes of impedance analysis of single cells are presented.

Dielectrophoresis is a well-known process to manipulate/separate a target cell type in complex populations and even capturing cells in an active electrical traps in single form. Detection of early stages of cancer diseases by cost effective LoCs is a good alternative to conventional methods where the point of care applications are strictly limited [1,5].

Impedance analysis of cancer cells is carried out by applying an AC signal to interdigitated electrode couple where a single cell is captured. Impedance measurement of interdigitated electrodes are recorded for selected frequencies and when a cell is trapped, an impedance shift occurs due to the physical/electrical conditions of an interfering biological cell [6,7]. Basic electric circuit systems are mathematically modeled depending on the electrode geometries and layer-by-layer physical structures of biological cells [6,7]. These models are used for calculation of electrical parameters of interior cell structures and can be used for further applications as in the case of maintaining higher efficiencies of DEP cell separation systems.
2. Design and Fabrication

Hydrodynamic traps for single cell analysis consist of three dimensional polydimethylsiloxane (PDMS) microchannel structures to maintain the cell within the trap area under continuous fluid flow. The idea behind the design is inspired from the study of Tan [8] where a two dimensional microfluidic channel consists of two potential paths (Path 1 and Path 2) for cells to follow under continuous fluid flow. The width and the height of the microchannel is close to the diameter of a cell to maintain the stream of cells in a linear form. Path 1 has a lower resistance and a narrow end to create a trap region. When a cell is trapped in Path 1, fluid flow is blocked or resistance gets too high for cells to pull in. Subsequent cells start to follow Path 2 until the next trap region appears. Single cell trap array is arranged by this loop of fluid flow throughout the microchannel. Due to the fabrication challenges of Tan’s design consisting of 5µm x 5µm narrow necks, we present a 3D PDMS microchannel which is shown in Figure 1 with a minimum feature size of 10µm. A narrow neck in Path 1 is created in “z” direction by limiting the channel height to 5µm in this region and the rest of the channel has a 15µm height. Conventional lithography processes are used to create such structures by aligning two mask to create 3D SU-8 3010 negative photoresist as a master mold for PDMS casting.

![Figure 1](image)

*Figure 1. a) A triangular trap region is placed to Path 1 b) The flow behaviour is simulated considering three different base angles (α=30°, 45°, 60°).*

Circular and triangular geometries with different dimensions and base angles are placed in Path 1 to investigate the most effective single cell trap area. The triangular trap area with a base angle of 30° has the best experimental outcome where the other geometries are most likely to trap more than one cell at a time. Distribution of fluid velocity is shown in Figure 2 and it can be seen that increasing the resistance of Path 2 by increasing the length is also a simple solution to achieve higher trapping efficiencies.

Further enhancement of preventing multiple cells trapping is done by placing a graded triangular trap area in Path 1 and can be seen in Figure 3. When a cell (1) is trapped in the acute angled region the subsequent cells (2) are cleared out of the trap area. Three different combinations are studied experimentally \((\alpha_1=30°, \beta_1=45°), (\alpha_2=30°, \beta_2=60°)\) and \((\alpha_3=45°, \beta_3=60°)\).

Interdigitated electrodes with a width of 15µm and a distance of 10µm are placed under a microchannel as an array. Ti microelectrodes with a 200nm thickness are fabricated on a 400nm thick AZ 1505 photoresist coated microscope slides by DC magnetron sputtering and lift-off processes. PDMS microchannel and microelectrodes are aligned under an optical microscope and plasma-activated bonding process applied.
Figure 2. Fluid velocity distributions for different Path 2 lengths ($\alpha=45^\circ$). Color bar: Fluid velocity (m/s).

Figure 3. Schematic view of the graded triangular trap design. A) Three different combinations are studied experimentally ($\alpha_1=30^\circ$, $\beta_1=45^\circ$), ($\alpha_2=30^\circ$, $\beta_2=60^\circ$) and ($\alpha_3=45^\circ$, $\beta_3=60^\circ$) B) When a cell is trapped in narrow region subsequent cells are cleared out of the trap area.

Figure 4. Schematic view of interdigitated electrodes (pink) placed under a microchannel (blue). The width of the electrodes is 15$\mu$m and the distance between each electrode couple is 10$\mu$m.
3. Experimental Results

Various trap geometries, circular and triangular, are studied in theory and experimentally with polystyrene particles and biological cells throughout our research but the most recent experiment results are presented in this paper. HCT116 (homosapiens colon colorectal carcin) and HEK293 (human embryonic kidney) cells 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 (1XPBS=15.70mS/cm, 0.1XPBS=1.87mS/cm). HCT116 cells are trapped in (α1=30°, β1=45°) angled triangular traps with a high efficiency and shown in Figure 5. Some of the cells are deformed in narrow neck regions due to the elastic structure of biological cells and the cells in red circles are not in touch with the microelectrodes where the impedance measurement is recorded with the cells in green circles.

HEK293 cells are also efficiently trapped using the same structure and the impedance shift is plotted for both cells and mediums based on the signal recorded before and after a cell trapped between electrode couples. 1Vpp signal with a 10-500 kHz frequency range is applied to microelectrodes and results are shown in Figure 6. It can be seen that the impedance shift varies for each cell line which can be used for further analysis or diagnosis applications. Divergence of impedance shift between cell lines are increases with decreasing the medium conductivity which makes low conductivity mediums favourable for diagnosing of cell lines from one another.
Figure 6. Impedance shift of HCT116 and HEK293 cell lines in 1XPBS (left side) and 0.1XPBS (right side) mediums. Note that $\Delta Z$ is increasing with decreasing the medium conductivity.

4. Conclusion

Our experimental results show that diagnosing of different cell lines in low conductive mediums is achievable using current single cell trap array structures. 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 calculating the physical/electrical properties of interior cell structures and the separation efficiency by DEP will be increased with gained knowledge of target cell lines. Chemical stimulation on a target cell using a specific type of drug could also be evidenced by the amount of these impedance shifts.

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