SINGLE CELL DIFFERENTIAL IMPEDANCE SPECTROSCOPY ANALYSIS USING HIGH DENSITY HYDRODYNAMIC CELL TRAPPING ARRAYS

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Abstract

We present a novel device for performing differential impedance spectroscopic analysis of single cells hydrodynamically captured in an array of trapping sites. The method combines the advantages offered by impedimetric analysis used for flow cytometry with the ability to capture multiple single cells and perform long-term transient and steady state analysis. We have tested the device by capturing single polystyrene beads and HeLa cells and measuring differential impedance responses.

Keywords: Single Cell Analysis, Differential Impedance Spectroscopy

1. Introduction

Impedance spectroscopy is by no means a novel method of analysis for tissues, cells and biomolecules[1]. Perhaps surprisingly, while other electrical methods of manipulation and sorting based on the dielectric properties of biological samples (dielectrophoresis, electrorotation) have found widespread utilization since the introduction of microfabrication and photolithography techniques, and in fact have greatly benefited from the inclusion in the μTAS and lab-on-chip paradigms, impedance spectroscopic methods have rarely been employed for the analysis of single cells, except for the case of flow cytometers.

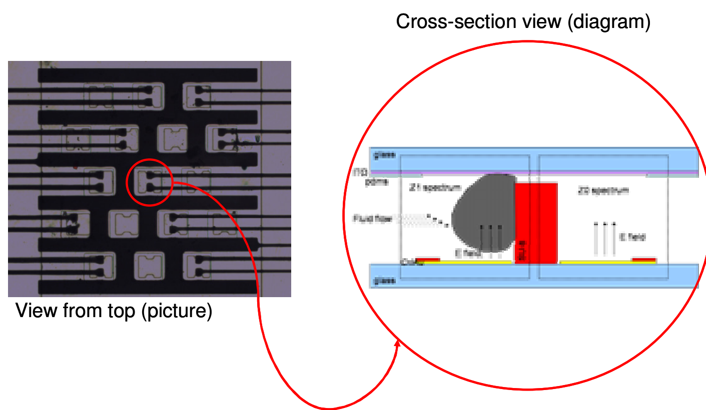

Figure 1 The differential nature of the measurements decreases sensitivity to local fluctuations in temperature and medium ionic strength and also normalizes the readings for differences in electrode shape, areas and coverage, as well as counterelectrode wetting conditions.

Impedance spectroscopy is a label-free, all electrical method of analysis which allows quantitative measurements of cell characteristics and changes in their membrane, cytoskeleton and nucleus elicited by the introduction of chemical compounds.

The impedance signal from a biological sample is resultant from the combination of electrical resistance and molecular and ionic polarization following excitation by an electric field. Different processes are responsible for the polarization of biological tissues and cells. In the frequency range between 10 kHz and 10 MHz, the effective permittivity (a measure of polarizability) of biological cells undergoes a dispersion, commonly attributed to the accumulation of charges at the interfaces between the membrane and the aqueous phases (i.e. the cytoskeleton and the bathing medium). Traditionally, impedance measurements have been performed on bulk quantities of cells [2]; these are not sensitive to the occurrence of rare events and do not allow the identification of outliers in the data; temporal averaging effects also affect measurements performed to an ensemble of cells making it impossible to record and identify fast kinetic events. To address these issues we have fabricated a device, which utilising metal electrodes aligned to SU-8 cell traps allows the analysis of single cells following their hydrodynamic capture. The trap design follows closely the one demonstrated by D.D. in [3]. Whereas impedance flow cytometers [4] only probe cells for a fraction of a second and at high frequencies, this device allows a full spectrum scan, and the analysis of a cell can take place continuously for as long as needed or desired.

2. Fabrication

The chip is fabricated employing standard microfabrication techniques: evaporation and patterning of metal electrodes via wet-etch process, spinning and photolithographic patterning of SU-8 negative photoresist. ITO coated slides (Sigma-Aldrich) were clamped to the SU-8 structures to form channels and function as a common ground electrode. Trapping sites were designed for differential measurements and optimally sized for capture of single cells.

3. Results and discussion

As a proof of concept we loaded the device with a solution of 1% polystyrene

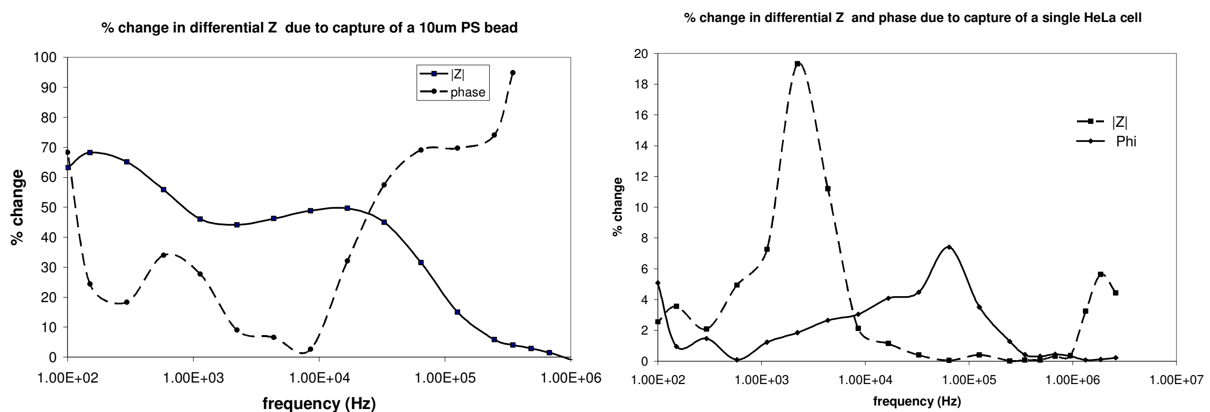

Figure 2 Differential Impedance Spectra for single bead (left) and single Hela cell (right). HeLa cell provokes a stronger differential phase response.

beads,10μm in diameter, and following their capture in the traps, measured the impedance response. The impedance following capture of a single bead shows a significant increase (>50%) over frequencies up to 10 kHz. We also loaded and detected presence of a single HeLa cell in a trap. As expected the response due to a single cell is weaker (changes averaging ~5% across the spectrum were detected, with a 20% peak at 5kHz) than the response elicited by a polystyrene bead of similar size, due to the dielectric differences between cells (which respond like shells containing a medium as conductive as the external electrolyte) and beads (which are solid spheres of dielectric material). In fact, the system is more sensitive to changes in the phase response at higher frequencies (10kHz < f < 1 Mhz) when looking at HeLa cells. Already Cheung et al. had shown in [3] that where changes in |Z| do not allow successful discrimination, it might be necessary to rely on phase response changes.

In the current design, the device employs a large ground electrode, which while simplifying the fabrication process and relaxing the alignment tolerances - negatively affects the sensitivity of the impedance readings. This occurs because the metal lines that connect the measuring electrodes to the peripheral pads for external connection also run parallel to the ground electrode, thus contributing to a parasitic capacitance which reduces the sensitivity of the device to changes in impedance. To address this issue and improve the sensitivity of the measurements, we plan to pattern the ground electrode, thus reducing its active area to the location of the trapped cells only.

4. Conclusions

We have presented a device for performing differential impedance measurements of single cells captured in an array of trapping sites. We have shown that we can successfully detect presence of single HeLa cells. We are currently improving the device to electrically monitor the transient behavior of cancer cells exposed to anti-tumoral agents by exploiting the changes induced in the dielectric properties of the membrane and cytoskeleton.

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References

[1] Foster KR, Schwan HP, Dielectric properties of tissues and biological materials - A critical review, Critical Reviews in Biomedical Engineering 17 (1): 25-104 1989
[2] K. Asami and T. Yamaguchi , Dielectric spectroscopy of plant protoplasts, Biophysical Journal, Vol 63, Issue 6 1493-1499, 1992
[3] Di Carlo D, Aghdam N, Lee LP, Single-cell enzyme concentrations, kinetics, and inhibition analysis using high-density hydrodynamic cell isolation arrays, ANALYTICAL CHEMISTRY, 78 (14): 4925-4930 JUL 15 2006
[4] Cheung K, Gawad S, Renaud P, Impedance spectroscopy flow cytometry: On-chip label-free cell differentiation,CYTOMETRY PART A 65A (2): 124-132 JUN 2005