EXAMINING CYTOTOXIC EFFECTS ON SINGLE CELLS USING AN IMPEDANCE SPECTROSCOPIC PLATFORM

Daniele Malleo*, J Tanner Nevill†, Dino Di Carlo†, Luke P. Lee†, Hywel Morgan*

*School of Electronics and Computer Science, University of Southampton, UK
†Department of Bioengineering, University of California at Berkeley

ABSTRACT

We present a microfluidic device for performing impedance spectroscopic analyses of the steady-state and time-transient response of single cells exposed to cytotoxic agents and drug compounds. This platform is capable of long-term label-free assays. Sample preparation steps normally associated with fluorescence analysis (fixing, staining and washing) are avoided. We analysed the transient impedance from single cells to study the kinetics of cell response to Streptolysin-O (SLO) on mammalian cells. The toxin was found to cause a gradual decrease in the impedance, which corresponded to an increase in membrane conductivity. Increasing concentrations of toxins caused a higher rate of change seen as an overall decrease in the impedance.

Keywords: Single Cell Analysis, Label Free Method, Impedance Spectroscopy

1. INTRODUCTION

Impedance spectroscopy is a label-free method of analysis which has the potential to allow quantitative measurements of cell characteristics, for example changes in properties of the membrane, cytoskeleton or nucleus elicited by chemical compounds. Dielectrophoresis is used for the manipulation and sorting of particles in micro-chips. The forces responsible for these phenomena are linked to the intrinsic dielectric properties of the biological particles. However, impedance analysis of cells in micro-systems is not yet part of the μTAS and lab-on-chip toolkit.

The platform described in this paper combines the advantages offered by impedance analysis [1], as used in micro-flow cytometry, with the ability to capture and monitor large numbers of single cells on a single device, so that long-term transient and steady state analysis can be performed. Cells are captured using the arrayed hydrodynamic single cell principle developed by Di Carlo et al [2]. The cells are held between two microelectrodes which are used to measure the impedance signal. The system enables the analysis of multiple single cells, allowing the detection of rare events and the identification of unique events. Temporal averaging is avoided because single cells are individually probed, individual responses and fast kinetic events can be recorded.

2. THEORY

The impedance signal from a cell sitting on or between electrodes is modulated by a number of factors, including the area of the cell in contact with the substrate and polarization processes. For a cell immersed in a high conductivity suspending buffer, measurements of the complex permittivity in the frequency range from 10 kHz and 10 MHz can be used to characterise the properties of the cell membrane. At lower frequencies the impedance is dominated by the high capacitance of the membrane. As the frequency...
increases the permittivity drops due to the short-circuiting of the membrane by the field. The impedance spectrum over the frequency range of 10 kHz to 1 MHz is therefore dominated by the cell membrane capacitance, but is also influenced by the cell membrane conductivity. This in turn is determined by the number and size of transmembrane channels. Calculations made using the single-shell model [3] show that the overall conductivity of the system is most sensitive to membrane conductivity. SLO is a toxic, cytolytic, and immunogenic protein, which represents the prototype of toxins that damage membranes through generation of large transmembrane channels, thus increasing membrane conductivity. Typical values for the membrane conductivity of a viable cell are $10^{-9} \text{S/m}$, the graphs (figure 1) shows that this has to increase to the order of $10^{-5} \text{S/m}$ (or ~$10^4$ SLO pores) to lead to measurable changes.

**Figure 1.** Plots of the permittivity and conductivity calculated using Maxwell’s mixture theory for a cell modelled as a single-shell particle. The plot was obtained for a volume of $20\times20\times20 \mu m$, with a homogenous electric field and a 10 $\mu m$ diameter cell, immersed in PBS. Membrane conductivity changes from $10^{-9} \text{S/m}$ to $10^{-5} \text{S/m}$.

**2. EXPERIMENTAL SYSTEM**

The chip is shown in cross section in figure 2 and contains an array of microelectrodes, two for each cell (for differential measurement) together with an hydrodynamic trap that confines the cell to the measurement region [2, 3]. The excitation frequency is applied through the top ITO (Indium Tin Oxide) electrode and the signal from each of the single cell electrodes is processed by an impedance analyzer connected via a multiplexer. MATLAB scripts control the multiplexing front-end, the impedance analyzer, and post-process the acquired data.

**Figure 2** (a) Cross section of the device showing the two microelectrodes on either side of the hydrodynamic obstruction which traps the cell. Fluid flows in from the left. A single
cell sits above one electrode, the other is used for differential measurement. The signal is applied through the top ITO electrode. (b) Photograph of a HeLa cell trapped in the device (c) The assembled device showing the fluid inlet ports on top.

3. EXPERIMENTAL

The device was loaded with HeLa cells suspended in PBS and the impedance continuously monitored from all the electrodes. 1ml of SLO toxin activated by 100mM DTT (DL-Dithiothreitol) was introduced into the device at concentrations ranging from 100 U/ml (0.09 μg/ml) to 10 kU/ml (9 μg/ml) to elicit changes in trapped single cells. As a control, the response to the introduction of 100mM DTT was also measured.

4. RESULTS

Figure 3 shows a time-evolution of the differential impedance (measured at 1kHz) for single HeLa cell after introduction of SLO. The control data demonstrates that the impedance of the cell remains constant with time. Exposure to 100 U SLO leads to a gradual decrease in the impedance, this change increases with increasing level of toxin as expected from the pore forming properties.

5. CONCLUSIONS

We have fabricated a device which successfully combines the advantages offered by impedance-based flow cytometry (ie label-free, electrical-based analysis) with the ability to capture multiple single cells and perform long-term transient and steady state analysis. We have shown that we can measure changes in membrane conductivity elicited by a pore-forming toxin, SLO.

REFERENCES


---

1 A unit (U) is defined as causing 50% lysis of a 2% red blood cell suspension in phosphate buffered saline, pH 7.4, after incubation at 37 °C for 30 min.