LABEL-FREE, HIGH THROUGHPUT ELECTRICAL DETECTION OF CELLS IN DROPLETS

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ABSTRACT

We present a label-free, impedance based cell in droplet detection system. Unlike other work [1,2], this system does not involve the use of valves or expensive labor-intensive cell labeling. The presented platform shows for the first time simple, fast and all electrical discrimination between empty and cell containing droplets. This enables the downstream selection of single-cell containing droplets, while the electrical measurement technique allows for easy integration.

KEYWORDS: Cell encapsulation, Droplets, Impedance, Label-free

INTRODUCTION

Droplet based microfluidics has become a standard platform for high throughput single-cell research and analysis. Therefore, single-cell encapsulation constitutes a significant part in droplet microfluidic research. However, many challenges still need to be tackled. As previously shown, deterministic encapsulation ensures up to 77% single-cell containing droplets [3]. However, a yield of 100% is not accomplished, therefore impedance based measurements are used to discriminate between empty and cell containing droplets. Moreover, viable and non-viable cells can be distinguished using low conducting (LC) buffer.

DESIGN

The microfluidic PDMS/Pyrex device has two main components, the droplet generator and the impedance sensor. The droplet generator has a flow focusing junction (depth 25 µm, width 40 µm, focus-width 25 µm). The continuous phase consisted of hexadecane with 1% w/w oil phase surfactant. The dispersed phase consisted of PBS (1.6S/m) or isoosmolar LC buffer (280 mM inositol, 0.1 mM calcium acetate, 0.5 mM magnesium acetate and 1 mM l-histidine), with or without NS-1 cells or beads. The conductivity of the LC buffer was adjusted using deionized water to 0.009 S/m. The impedance sensor comprises two planar platinum electrodes with an inter-electrode distance of 30µm patterned on glass. The impedance was measured at 100 kHz, and $6V_{pp}$ at a sample rate of 899 Hz using the HF2IS impedance spectroscope (Zurich Instruments) with a homemade current amplifier (fig1).

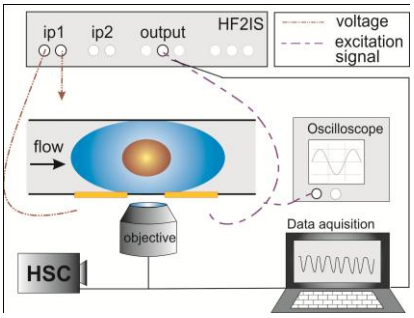

Figure 1: A schematic overview of the measurement set-up. The microfluidic chip is connected, via a homemade current amplifier, to the impedance analyzer (HF2IS), which is connected to a PC and an oscilloscope. Visual inspection of the set-up is performed via an inverted microscope connected to a high speed camera (HSC).

RESULTS

The frequency behavior of the microfluidic chip with LC buffer and PBS, with and without cells was determined, to ensure that the electrical impedance measurements were performed at a frequency within the resistive plateau. Figure 2A and B shows the averaged results of 50 individual impedance measurements of a chip filled with PBS or LC buffer for frequencies from 1000 Hz to 40 MHz. These data propose that when using LC buffer, the behavior of viable cells with respect to the LC buffer alone is opposite to the behavior of non-viable cells. The decrease in resistance of viable cells in LC buffer, can be explained by the presence of the conducting cytoplasm, resulting in an increase in conductivity at the measured frequency, which was confirmed by Matlab simulations (fig2C) using Maxwell-Wagner theory. A simplified equivalent circuit model for three different situations is given in figure 3.

978-0-9798064-5-2/µTAS 2012/$20©12CBMS-0001

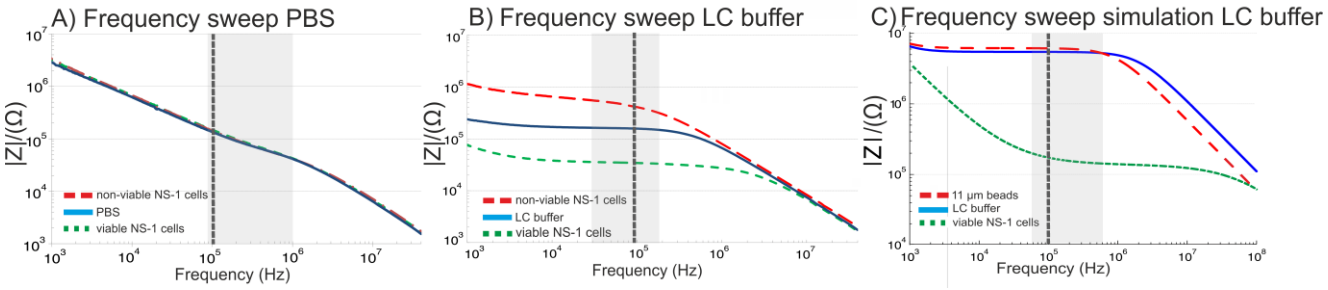

Figure 2: A and B) The measured frequency behavior of the microfluidic chip. The lines in the graph show the average of 50 measurements obtained with the impedance/gain phase analyzer using LC buffer with or without (non)viable NS-1 cells. The shaded area indicates the measurement frequencies which have been studied and the dashed line represents the frequency used during all subsequent experiments C) Simulation of the frequency response of the real electrical impedance of the equivalent circuit model with viable NS-1 cells, beads(representing non-viable cells) and only LC buffer.

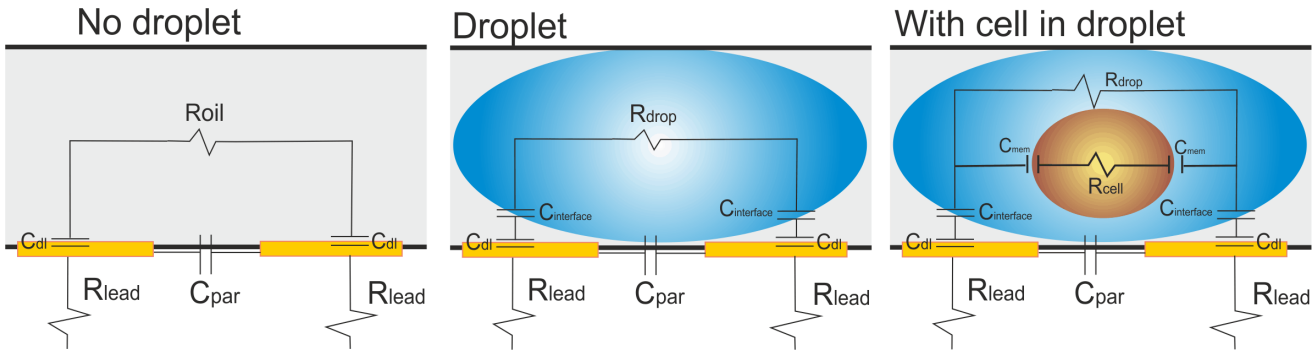

Figure 3: Simplified equivalent circuit model of the microfluidic device w/o droplet, with droplet and with cell in droplet. In all situations, there are two double layer capacitances (C_{dl}), caused by the electrode-fluid interface, a parasitic capacitance (C_{par}) and a lead resistance (R_{lead}). The passage of an empty droplet adds two capacitances to the model and when the droplet contains a cell, an additional circuit for the cell is implemented. This is represented by the addition of two capacitances for the oil-buffer interface at the droplet ($C_{interface}$), a resistance of the droplet fluid (R_{drop}) and an equivalent circuit model for a cell. The capacitances in this simplified model of the cell are the cell membrane (C_{mem}) and the resistance of the cell interior (R_{cell}).

With our device, droplets containing LC buffer or PBS were detected up to 475Hz. NS-1 cells suspended in LC buffer were detected and can be differentiated regarding viability (fig4). Viable NS-1 cells show negative peaks, whereas non-viable cells show positive peaks. Furthermore, polystyrene beads suspended in LC buffer behave similar to non-viable cells (fig4). When a mixture of viable and non-viable NS-1 cells were suspended in PBS, only positive peaks were observed (fig4), of which the height of the individual peak area corresponded to cell size (not shown). Hence, by using LC buffer it is possible to differentiate between viability based on the peak appearances.

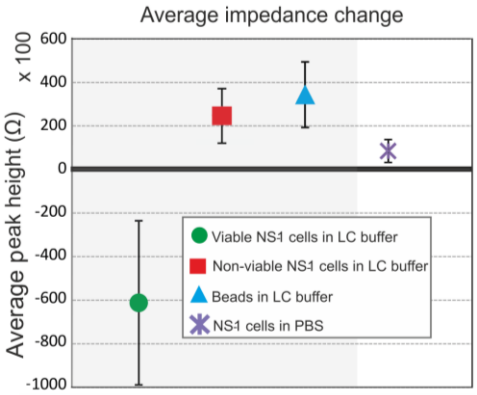

Figure 4: Impedance change of viable (n=68) and non-viable NS-1 cells (n=154), 11 µm sized beads all in LC buffer (n=15) and impedance change of viable and non-viable NS-1 cells in PBS (n=37). The shaded area represents data from LC buffer.

Next, cells were encapsulated in LC buffer. Since, the LC buffer is still more conducting than the continuous phase (oil), the impedance signal of empty droplets decreased, resulting in negative peaks. Cell containing droplets generated an increasing negative peak, in the real part of the impedance measurements, compared to empty droplets (fig5), which is in correspondence with our observation. Cell containing droplets can be detected up to a droplet frequency of 60Hz.

Future experiments are focused on increasing the operating frequency to several kilohertz, using a dedicated lock-in-amplifier and discriminate between non-viable and viable cells in LC droplets. Furthermore, we want to detect cells in PBS droplets and perform downstream selection of cell containing droplets.

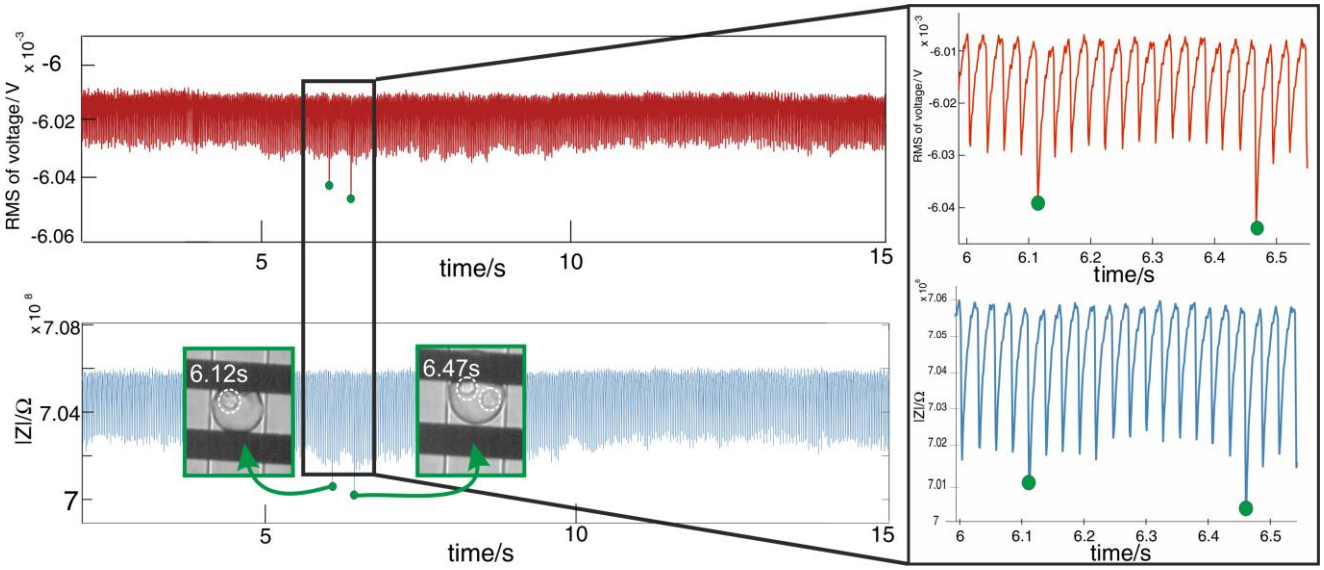

Figure 5: NS-1 cell in droplet detection using RMS voltage signal and the final impedance change. NS-1 cells in LC buffer were detected at a droplet frequency of 40 Hz. F_{act} = 100kHz sample rate is 899Hz and 6 V_{pp}.

CONCLUSION

This is the first time that label-free detection of cells in droplets is shown. The device enables us to measure the individual volume, frequency and even content of the droplets. Moreover, cells can be discriminated based on their viability.

ACKNOWLEDGEMENTS

Financial support from ERC (eLab4life project) and technical assistance of J.G. Bomer and P.M. ter Braak are gratefully acknowledged.

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