32nd URSI GASS, Montreal, 19-26 August 2017

Dielectric Study of Biological Phenomena at the Single Cell Level: Electroporation and Starvation

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Abstract

In this paper we present the use of a dielectrophoresis (DEP) cytometry technique to study changes in single cells upon application of a particular stimulus. In one case we measure single CHO cells immediately after exposure to pulsed electric fields. Our results show a significant change in the dielectric response of cells after application of sufficiently strong pulses. In another study we investigate the effect of nutrient deficiency on CHO cells. Many individual cells are measured as they undergo starvation due to lack of nutrients in their medium. Our DEP measurement results, after 48 hours of starvation, show a substantial change in the dielectric response that correlates with the viability of the cells. By the examples we present in the paper, we demonstrate the potential of our DEP cytometry technique in applications such as monitoring the effect of drugs or developing optimal protocols for cell electroporation.

1. Introduction

Label-free and non-invasive dielectric study techniques has attracted interest as an emerging modality for studying biological phenomena. Studies at the cellular level, rather than measuring the average response of a bulk sample, has the advantage of giving insight into the behavior of each cell without the influence of neighboring cells and aggregation effects. Moreover, studying individual cells can provide information about the sub-populations (cells at different physiological states) in a sample.

Dielectrophoresis, translation of polarizable particles in a non-uniform electric field, is a well-known dielectric technique capable of measuring single cells. It has previously been employed for separation, characterization, and manipulation of various type of biological cells [1]. In this paper we employ a dielectrophoresis cytometry technique to dielectrically study single Chinese hamster ovary (CHO) cells as their state changes upon application of an internal or external stimulation. We show measurement results for two distinct stimulations: electroporation and nutrition deprivation. Both studies demonstrate the ability of dielectric-based techniques as a label-free and non-invasive modality to investigate biological phenomena at a cellular level.

2. Experimental Set-up

2.1 Dielectrophoresis

Dielectrophoresis (DEP) is the induced motion of a polarizable particle in a non-uniform electric field. The DEP force exerted on the cell is given by [2]

$$\vec{F}_{DEP} = \frac{3}{2}\varepsilon_e V_c Re\{K_{CM}(\omega)\}\vec{\nabla}\left(E_{rms}^{DEP\,2}\right), \qquad (1)$$

where ε_e is the medium permittivity, V_c is the cell volume, and E_{rms}^{DEP} is the rms value of the electric field at the center of the cell. $Re\{K_{CM}\}$ is the real part of the Clausius-Mossotti factor given by

$$K_{CM} = \frac{\tilde{\varepsilon}_c - \tilde{\varepsilon}_e}{\tilde{\varepsilon}_c + 2\tilde{\varepsilon}_e}. \qquad (2)$$

In (2) $\tilde{\varepsilon}_e$ and $\tilde{\varepsilon}_c$ are the complex permittivity of the media and the cell, respectively. The DEP force is frequency dependent through $Re\{K_{CM}\}$ and is directed with (pDEP) or against (nDEP) the gradient of the square of the electric field depending on the sign of $Re\{K_{CM}\}$.

2.2 DEP Cytometer

We employ a dielectrophoresis cytometry technique to study cells as they undergo physiological changes due to internal or external stimulations such as nutrition deprivation and electroporation. The details of the DEP measurement technique has been described elsewhere [3]. Briefly, an array of electrodes are embedded at the bottom of a microfluidic channel which are employed to sense single cells and actuate them by a DEP force. As shown in Fig. 1a, two sets of sensing electrodes, S_1 and S_2, located on the sides of a DEP actuation electrode, A, capacitively sense the altitude of a cell in the channel. This is achieved by using a microwave interferometer (operating at 1.5 GHz) with a sensitivity of less than 0.1 aF for a detection bandwidth of 42 Hz. The output of the interferometer is a signal, S(t), with two peaks corresponding to the two sensing electrodes (example signal shown in Fig. 1b). The amplitude of each peak is proportional to the capacitance change of the corresponding sensing electrode due to the cell and subsequently its altitude in the channel. The interferometer has been described in detail elsewhere [4]. As a cell passes over the electrode array the DEP force,

generated by applying a proper voltage to the actuation electrode, causes the vertical movement of the cell, see Fig. 1b. This leads to a sensing signal with its second peak larger (pDEP) or smaller (nDEP) than the first one. A parameter, force index, defined as $(P_2 - P_1)/P_1$ is used to quantify the measured signals. The magnitude and sign of force index is an indication of the intensity and direction of the DEP force acting on a cell and hence its dielectric properties. The DEP voltage applied to the actuation electrode is an 8 V_{pp} sinusoidal voltage with frequency chosen to measure a particular cell property.

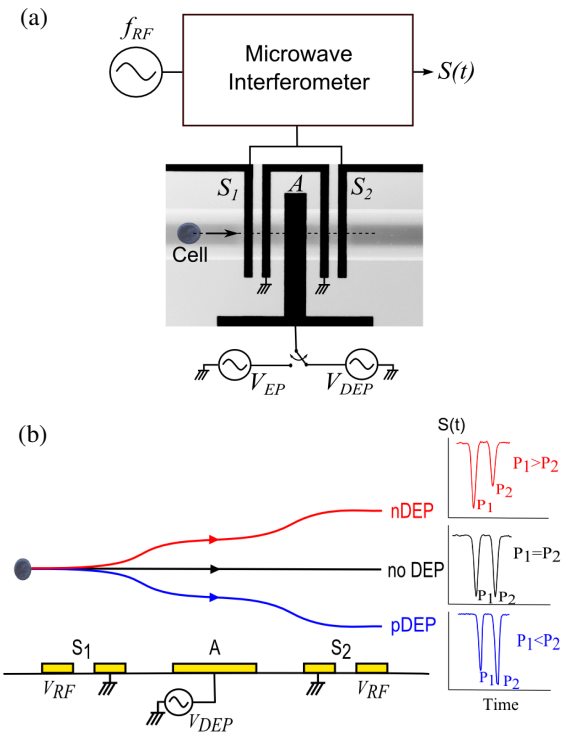

Figure 1. (a) Micrograph of the sensing, S_1 and S_2, and actuation, A, electrode array in the microfluidic channel. Cells are sensed in the channel using a microwave interferometer. By applying a proper voltage to the actuation electrod, cells are actuated by a DEP force (for measuring their dielectric response) or stimulated by pulsed electric fields (for electroporating cells, see the Results section). (b) Schematic trajectory of a cell experiencing pDEP, nDEP, or no DEP as itr passes over the electrode array. Example measured signals by the microwave interferometer, S(t), for single CHO cells is presentd in the right pannel for three cases of pDEP (P_1<P_2), noDEP (P_1=P_2), and nDEP (P_1>P_2).

3. Results and Discussion

We employed our DEP cytometry technique to investigate changes in the dielectric properties of single CHO cells in two separate experimental studies: one with cells exposed to pulsed electric fields (causing electroporation of cells) and the other with cells suspended in a medium without essential nutrients (glucose and glutamine). In both cases the cells were suspended in a medium with conductivity 0.17 S/m for the DEP measurement.

In the electroporation experiment a single cell was selected and shuttled back and forth over the electrode array in the microfluidic channel for approximately 5 minutes, similar to the approach suggested in [5]. First the force index of the untreated cell was measured for several passes over the electrodes. Then a train of pulsed voltages with the desired intensity and duration was applied to the actuation electrode to electroporate the cell as it traveled one pass over the electrodes. Afterwards, the DEP voltage was applied to the actuation electrode and the cell's force index was measured immediately after the pulse exposure for approximately 3 minutes. Fig. 2 shows the measured force indices over time for cells exposed to eight 100 µs duration pulses with intensity 1.8 kV/cm (Fig. 2a) and 0.7 kV/cm (Fig. 2b). It is evident that while the pulses of 0.7 kV/cm were not sufficiently strong to affect the cell pulses of 1.8 kV/cm had significant impact on the cells dielectric properties. Pulses stronger than 1 kV/cm has been shown in literature to cause measurable effects on cells [6].

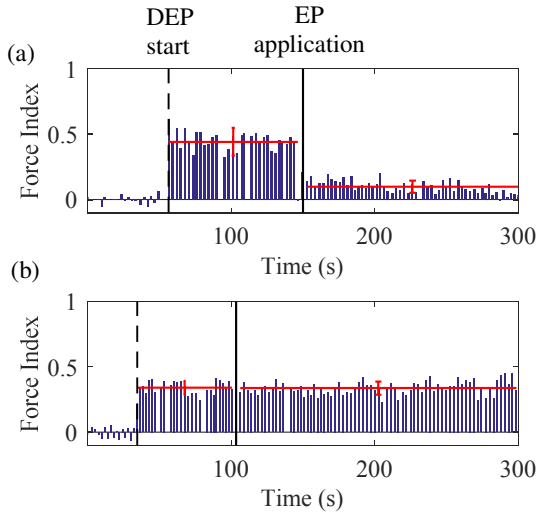

Figure 2. Measured force indices for two CHO cell shuttled back and forth over the electrode array in the microfluidic channel and subjected to eight 100 µs pulses with amplitude (a) 1.8 kV/cm and (b) 0.7 kV/cm. Each bar represents one pass of the cell over the electrodes. The red lines indicate the average with the error bars being the standard deviation. Before the DEP application (shown by a dashed black line) the force index is approximately zero. After DEP application the cell experiences a strong pDEP force. After electroporation (shown by a solid black line) the intensity of the pDEP force decreases for the cell exposed to pulses of 1.8 kV/cm while it remained unchanged for the one exposed to 0.7 kV/cm.

In our second experimental study, we investigated the effect of starvation on the dielectric properties of cells. This is important as shortage in nutrients is the primary

cause for triggering apoptosis in batch cultures. In this study we measured the DEP response of CHO cells cultured in a growth medium depleted from glucose and glutamine over a 48 hour period. In order to obtain information about the cells populations as they undergo starvation, the DEP response of many individual cells were measured as they passed over the electrode array and actuated by a DEP force (note that in this study each cell's response is measured only once. Unlike the electroporation study where one cell was shuttled back and forth and measured several times). Fig. 3 shows the histogram of measured force indices for cells after 4 and 48 hours culturing in the nutrient depleted medium. The results show that in the beginning a single population of cells is observed with positive force indices (experiencing pDEP). After 48 hours, a large population of cells demonstrate negative force indices (experiencing nDEP), while a small population still remain with positive force indices. We have shown in our previous study [7], on apoptosis of cells, that the force index of zero is a suitable threshold for determining the viability of cells. This was determined by comparing the results of the DEP cytometer with the standard biological assays [7]. Therefore, the results of Fig. 3 demonstrates a substantial change in the viability of cells from 98% to 24% after 48 hours of nutrient deprivation. Once the force index response has been determined, simulations can be employed to the cell's dielectric parameters such as membrane, cytoplasm, and nucleus permittivity and conductivity [8].

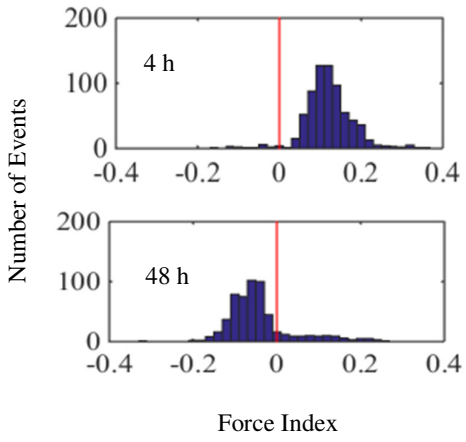

Figure 3. Histogram of measured force indices for CHO cells after 4 and 48 hours culturing in a growth medium without glucose and glutamine. In the beginning mejority of cells are viable while after 48 hours the majority are non-viable.

4. Conclusions

Using a dielectrophoresis cytometry technique we demonstrated the application of dielectric measurements in studies of biological phenomena. In one application we measured changes in the DEP response of cells after exposure to sufficiently strong pulsed electric fields. This has application in optimizing the process of electroporation (e.g. pulse parameters) for specific applications. In another study we showed how the viability of cells changes under control nutrient deprivation. This has application in monitoring the effect of drugs and developing personalized treatments. Both these studies show the ability of label-free and non-invasive dielectric-based techniques for investigating biological systems.

5. Acknowledgements

The authors thank the Natural Sciences and Engineering Research Council (NSERC) of Canada, the Canada Foundation for Innovation (CFI), Canadian Microelectronics Corporation (CMC) Microsystems and the Province of Manitoba for financial support of this research.

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