MEASUREMENT OF ELECTROPORATION INDUCED CHANGES IN THE DIELECTRIC RESPONSE OF SINGLE CELLS

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ABSTRACT

Electroporation affects the dielectric properties of cells by creating pores in their membrane which allow transport of ions in and out of cells. Dielectric characterization techniques are powerful tools to investigate the time evolution of electroporation induced changes. Here we employ a dielectrophoresis (DEP) technique to study the effect of electroporating pulse intensity on the temporal response of individual cells. Experiments on CHO cells demonstrate a shorter response time for cells exposed to higher intensity pulses.

KEYWORDS: Cell conductivity, Dielectric properties, Dielectrophoresis, Electroporation, Microfluidic, Single cell

INTRODUCTION

By applying a sufficiently strong pulsed electric field to a biological cell transient pores are created in the cell membrane, electroporation [1], through which charged molecules can be transported, subsequently changing its physiological state [2]. Dielectric spectroscopy has previously been used to study the of electroporation a population of cells a few minutes after exposure [3-4]. In this study we employ a microfluidic device to electroporate a single biological cell using a high intensity pulsed electric field (PEF) and simultaneously study induced changes in its dielectric properties by dielectrophoresis [5]. Using this system we perform a study of the time constant of changes in the internal conductivity of single cells a few seconds after exposure to PEF and its relationship to pulse intensity.

THEORY

A cell in a non-uniform electric field will be subjected to a force, dielectrophoresis (DEP), expressed as [6]

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

where ε_e is the permittivity of the medium, V_{cell} is the volume of the cell, and E_{rms} 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, which is a measure of the cell's polarizability with respect to the surrounding media at a frequency ω, 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 defined as $\tilde{\varepsilon} = \varepsilon - j\sigma/\omega$. The DEP force is oriented along (pDEP) or against (nDEP) the gradient of the square of the electric field depending on the sign of $Re\{K_{CM}\}$. Simulated $Re\{K_{CM}\}$ for various dielectric parameters of a cell (not shown here) indicates that at frequencies around 10 MHz, $Re\{K_{CM}\}$ is primarily influenced by changes in the cell internal conductivity. Therefore, continuous monitoring of changes in the response of a cell to DEP actuation at 10 MHz after electroporation can reflect ongoing changes in its internal conductivity. Fig. 1 illustrates the frequency dependent behavior of $Re\{K_{CM}\}$ for a typical CHO cell with three different values of cytoplasm conductivity in a medium of conductivity 0.17 S/m. It shows that changing the cytoplasm conductivity from 0.32 (typical for mammalian cells) to 0.17 (medium conductivity in our study) dramatically affects $Re\{K_{CM}\}$ and consequently the DEP force acting on the cell.

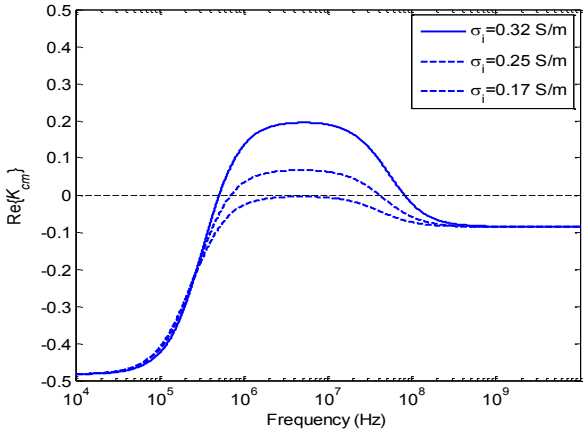

Figure 1: $Re\{K_{CM}\}$ vs. frequency for a CHO cell with three different values of cytoplasm conductivity in a medium of conductivity 0.17 S/m (a single shell model with $\varepsilon_{membrane} = 6.8$, $\sigma_{membrane} = 3 \times 10^{-6}\, S/m$, $\varepsilon_{cytoplasn} = 60$, $d_{membrane} = 5\ nm$, and $R_{cell} = 6.5\ \mu m$ is used.)

EXPERIMENT

Our system, see Fig. 2(a), uses a microwave interferometer to sense the change in the vertical position of a cell as it passes over a set of sensing electrodes, S_1 and S2, in the microfluidic channel. The interferometer has been described in [7]. As a cell passes over sensing electrodes a two peak signature, S, is registered, P_1 and P_2. The middle electrodes, A, are used to apply PEF to cells as well as actuate them with a DEP force. The DEP response is obtained by applying a 10 MHz sinusoidal voltage to the actuating electrodes. As a result of DEP forces acting on a cell in a low conductivity media, the cell is pulled towards the electrodes, registering a signature with asymmetric peaks (P_2>P_1), Fig 2(b). Since the DEP force is related to $Re\{K_{CM}\}$, the vertical displacement is also dependent on $Re\{K_{CM}\}$ and thus the dielectric properties of the cell. To quantify this we use force index parameter, $\varphi=(P_2-P_1)/(P_2+P_1)$, which is a direct indication of change in K_{CM}.

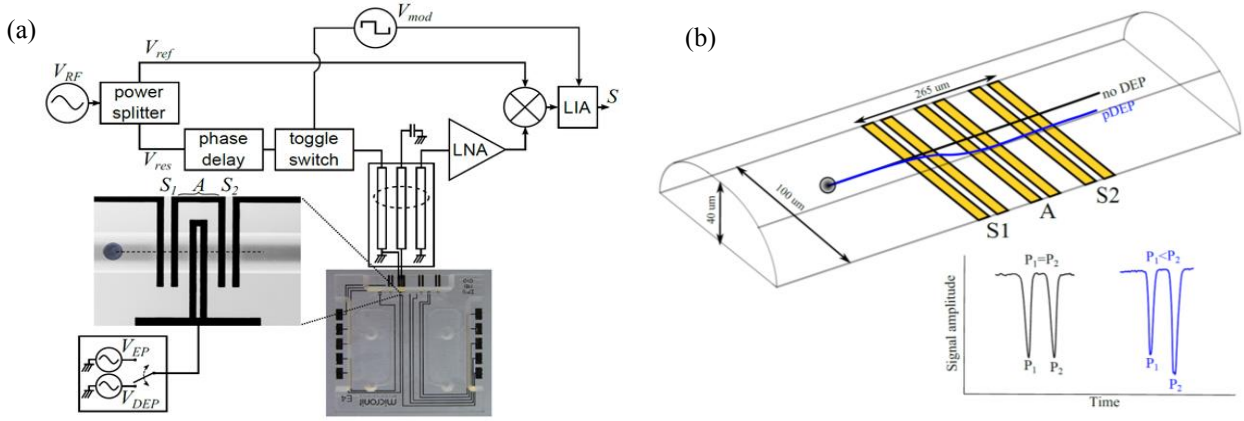

Figure 2: (a) Microwave interferometer connected to the microfluidic device to simultaneously electroporate and study the DEP response of biological cells. (b) Schematic representation of a cell trajectory in the microfluidic channel without and with positive DEP actuation and the corresponding sensed signatures. With no DEP the signal has two similar peaks whereas with positive DEP actuation the second peak is larger.

RESULTS AND DISCUSSION

We performed experiments on single Chinese Hamster Ovary cells suspended in a media with conductivity of 0.17 S/m. The experimental process is; *(i)* the signature and φ of a cell without and with DEP actuation is recorded by moving the cell back and forth over the electrodes; *(ii)* fifteen 5 microsecond duration pulses with repetition rate 200 Hz are applied to the cell. The intensity of the pulse is dependent on the cell height, which is determined by the first peak amplitude of the signature; *(iii)* the cell DEP response is recorded a few seconds after PEF exposure. φ is recorded for several minutes. Fig. 3 shows φ vs. time for 5 cells exposed to pulses with different intensities. The plots show noticeable change in the DEP response of cells within seconds after electroporation which simulation indicates is primarily related to the cell cytoplasm conductivity for our applied DEP frequency (10 MHz). It also demonstrates that change in the cytoplasm conductivity occurs gradually after PEF exposure and its time constant is dependent on the pulse intensity. Table 1 shows the time constant associated with force index change as obtained by fitting exponential curves to the data. It indicates that cytoplasm conductivity changes faster for stronger pulses. Decrease in the cell internal conductivity may occur after electroporation in our experiment as the cell media is less conductive than the cytoplasm. Transport of ions from the cytoplasm into the external media leads to lower cytoplasm conductivity and consequently smaller $Re\{K_{CM}\}$.

Table 1. Time constant of change in cell dielectric properties for cases of Fig. 3 obtained by exponential curve fitting

Case in Fig. 3	Time constant of the fitted exponential curve (s)	Relative pulse intensity with respect to case (a)
a	125	1
b	26	1.05
c	25	1.07
d	23	1.08
e	4	1.18

CONCLUSION

Studying induced changes in the dielectric properties of cells after electroporation can provide valuable information about the process of electroporation and the time constant of occurring changes. In this study we employed a dielectrophoresis technique to investigate the effect of the electroporating pulse intensity on the temporal dielectric response of the exposed cell, which simulation alludes is mainly due to changes in the cell internal conductivity. Experiments on CHO cells indicate that the cytoplasm conductivity changes faster for more intense pulses.

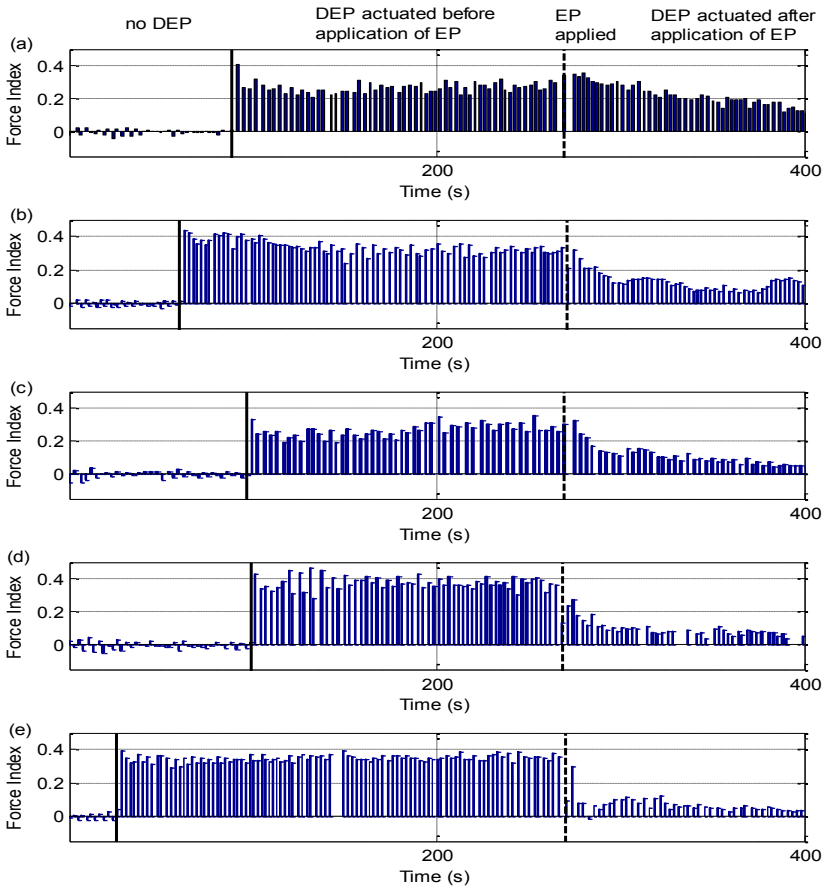

Figure 3: Force index vs. time for five CHO cells before and after exposure to PEF with different pulse intensities increasing from (a) to (e). In each case the force index is shown for three states: (i) no DEP actuation to verify that φ is close to zero (before the solid black line), (ii) DEP actuation before application of PEF to record the untreated cell DEP response (between the solid and dashed black lines), and (iii) DEP actuation after application of PEF to study the effect of electroporation (after the dashed black lines). The plots show noticeable change in the DEP response of cells within seconds after electroporation. It also shows a shorter response time for cells exposed to more intense pulses.

ACKNOWLEDGEMENTS

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