Dielectrophoresis study of temporal change in internal conductivity of single CHO cells after electroporation by pulsed electric fields

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Applying sufficiently strong pulsed electric fields to a cell can permeabilize the membrane and subsequently affect its dielectric properties. In this study, we employ a microfluidic dielectrophoresis cytometry technique to simultaneously electroporate and measure the time-dependent dielectric response of single Chinese hamster ovary cells. Using experimental measurements along with numerical simulations, we present quantitative results for the changes in the cytoplasm conductivity of single cells within seconds after exposure to 100 μ s duration pulsed electric fields with various intensities. It is shown that, for electroporation in a medium with conductivity lower than that of the cell's cytoplasm, the internal conductivity of the cell decreases after the electroporation on a time scale of seconds and stronger pulses cause a larger and more rapid decrease. We also observe that, after the electroporation, the cell's internal conductivity is constrained to a threshold. This implies that the cell prevents some of the ions in its cytoplasm from diffusing through the created pores to the external medium. The temporal change in the dielectric response of each individual cell is continuously monitored over minutes after exposure to pulsed electric fields. A time constant associated with the cell's internal conductivity change is observed, which ranges from seconds to tens of seconds depending on the applied pulse intensity. This experimental observation supports the results of numerical models reported in the literature. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4975978>]

I. INTRODUCTION

Electroporation (EP) is the formation of conductive pores in the membrane of biological cells as a result of exposure to intense pulsed electric fields (PEFs). The cell membrane polarizes in a strong electric field resulting in an increase in the electric potential across it. Above a certain threshold, the lipid bilayer molecules of the membrane rearrange themselves and form pores through which impermeable molecules and ions can pass in and out of the cell.^{1–3} The phenomenon has various biological and biomedical applications such as electrogenetherapy, electrochemotherapy, bacterial decontamination, and irreversible electroporation of cancerous cells.^{4–19} Different application methodologies employ pulses with various durations and intensities ranging from milliseconds to sub-nanoseconds and sub-kV/cm to MV/cm.²⁰ Long duration pulses (microseconds to milliseconds) can result in a host of physiological effects including necrosis, reversible or irreversible permeabilization.²⁰ Electroporation using nanosecond duration pulses, such that the pulse duration is less than the cell membrane charging time-constant, has shown to affect the cell's internal organelles and has been related to the induction of apoptosis.^{21–29}

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Besides the conventional methods of studying electroporation such as dye exclusion techniques, fluorescent cytometry, and patch-clamp techniques, dielectric based methods are attracting interest as a new label-free and non-invasive modality to investigate this phenomenon. The dielectric properties of a cell exposed to pulsed electric fields (PEF) are affected during and after electroporation due to the changes in the membrane structure and ion/molecule transport through the created pores. The conductivity of the membrane is significantly higher during electroporation since the created pores are much more conductive than the intact regions of the membrane.^{2,30–32} Further, the influx and efflux of ions affect the dielectric properties of the cell cytoplasm.^{33–35} Therefore, electroporation and its subsequent physiological effects on a cell can be studied by monitoring the changes in the cell dielectric properties after electroporation. The necessity of considering a dynamic membrane conductivity in modeling the response of cells to a single electroporating pulse has previously been discussed in several studies.^{36–41} Cytoplasm conductivity is also a parameter that can be dramatically influenced by electroporation. In multiple pulse applications (with slow repetition rate), this can change the charging time constant of the cell's internal compartments and impact the electroporation process.

Dielectric based techniques have been employed in several studies to investigate the electroporation of a bulk suspension of biological cells. Changes in the conductivity of a pellet of cells before and after electroporation with microsecond and millisecond pulses have been reported in other studies.^{32,42} A frequency-domain impedance measurement has been used to study the change in the conductivity of a suspension of cells due to electroporation.⁴³ A time-domain reflectometry (TDR) has been employed to study the changes in the electrical properties of cells subjected to microsecond and nanosecond pulses.^{44–47} Studying the electroporation of cells subjected to microsecond pulses using a dielectrophoresis (DEP) technique, based on monitoring the cross-over frequency or by field-flow fractionation, has been reported in the literature.^{48–51} Bulk electroporation and studying the response of a population of cells in suspension provide the overall response of the distribution of cells with different sizes, shapes, and possibly physiological states. There have not been many studies on single cells. Single cell studies, however, have the advantage of being able to examine the response of a cell with a known shape and size in a particular phase and without the influence of neighboring cells or aggregation effects. In a recent study, the dielectric properties of individual cells before and after electroporation have been investigated using an electric impedance spectroscopy technique.⁵² In the study, cells were exposed to high intensity continuous sinusoidal electric fields as they passed through a set of poration electrodes. Electroporation of single cells subjected to high intensity PEFs has also been studied using microwave impedance spectroscopy⁵³ and DEP based methods.⁵⁴

Dielectrophoresis, translation of polarizable particles in a non-uniform electric field, is a well-known phenomenon employed in the dielectric study of biological cells. DEP based techniques have previously been used to study stem cells,⁵⁵ differentiate different types of cells in blood analysis,⁵⁶ separate viable and non-viable yeast cells,^{57,58} isolate circulating tumor cells from a blood sample,⁵⁹ trap cells for lysing with electroporation,^{60–62} and detect early stage apoptosis in Chinese hamster ovary (CHO) cells in batch culture.^{63,64} Reviews on the theory and applications of DEP can be found elsewhere.^{65,66}

In this paper, we present a study on changes in the cytoplasm conductivity of single Chinese hamster ovary (CHO) cells immediately (within a few seconds) after electroporation employing a DEP cytometry technique. A microfluidic chip with an array of sensing and actuating electrodes embedded in its microfluidic channel was used to detect single cells, electroporate them, and apply a DEP force to them. To achieve the required sensitivity for the detection and dielectric characterization of single cells, we used a resonator-enhanced microwave interferometer, which measures the microwave frequency differential impedance of sensing electrodes as a cell passes over them. Detection at microwave frequencies, rather than at frequencies below 200 MHz, reduces the effects of ionic current flow, fluid-membrane interfacial polarization, large induced membrane potentials, and electrode polarization. As a single cell was continuously shuttled back and forth over the electrode array, a DEP force, at a frequency dominantly sensitive to the cytoplasm conductivity, translated the cell in the microfluidic channel before

and after exposure to PEFs. The DEP induced displacement of the cell was then related to its Clausius-Mossotti factor and subsequently cytoplasm conductivity by numerical simulations. The dynamic response of the cytoplasm conductivity change of several individual cells subjected to microsecond pulses with various intensities is reported (from seconds after electroporation to minutes).

II. MATERIALS AND METHODS

In order to study the effect of electroporation on the ionic content of cells at a cellular level, we employed a dielectrophoresis cytometry technique. As shown in Fig. 1, this consists of a microfluidic chip with an embedded electrode array, which can be used to simultaneously apply pulsed electric fields to a cell and measure its dielectric response over several minutes. Individual cells were selected as they flowed in the microfluidic channel and were shuttled back and forth over the electrode array. Each cell was then exposed to PEFs, and its DEP response was measured before and immediately after the pulse exposure. A resonator-enhanced microwave interferometer, which measures the capacitance change due to the cell, was employed to study the DEP response. Fig. 1 shows the block diagram of the microwave interferometer and the configuration of the electrodes in the microfluidic channel (the details of the system are discussed in Section II B). The electroporation induced changes in the cell's DEP response was translated into the changes in its cytoplasm conductivity using numerical simulations.

A. Cell culture

The Chinese Hamster Ovary (CHO) cell line (CHODG44-EG2-hFc/clone 1A7) used for this study was kindly provided by Yves Durocher of the NRC, Canada. The cells were maintained by passaging them every 2–3 days and growing them in 250 ml baffled shaker flasks (VWR International, Radnor, PA) at 120 rpm in an incubator at 37 °C with a 10% CO₂ overlay. The culture medium used was BioGro-CHO serum-free (BioGro Technologies, Winnipeg, MB) supplemented with 0.5 g/l yeast extract (BD, Sparks, MD), 4 mM GlutaMax I (Invitrogen, Grand Island, NY), and 1 mM glutamine (Sigma, St. Louis, MO). For this study, a sample of cells was taken from the shaker flask and centrifuged at 377 g for 1 min. The cell pellet was then re-suspended in fresh growth and low conductivity (~ 0.067 S/m) medium [22.9 mM

FIG. 1. (a) Schematic of the resonator-enhanced microwave interferometer employed to capacitively sense single cells in the microfluidic channel and estimate their altitude at sensing electrodes, S_1 and S_2 . DEP or EP signals are applied to the actuation electrode, A . (b) A typical measured signal, $S(t)$, for a single CHO cell as it crosses S_1 and S_2 at an altitude approximately 16 μm above the electrodes (no DEP or EP signals are applied). The capacitance change of the sensing electrodes due to a typical CHO cell is approximately 30 aF. The interferometer sensitivity is typically less than 0.1 aF.

sucrose (Sigma), 16 mM glucose (Fisher), 1 mM CaCl_2 (Fisher), and 16 mM Na_2HPO_4 (Fisher)]⁷⁷ at a ratio of 1:15 to reach a final conductivity of ~ 0.17 S/m as determined using a conductivity meter (Orion 3-Star Plus; Thermo Scientific, Waltham, MA). While re-suspending the cell pellet, the sample was diluted to a final concentration of 5×10^4 cells/ml.

B. Microfluidic system for DEP measurement and electroporation

Our microfluidic chip, fabricated by Micronit Microfluidics (Enschede, The Netherlands), consists of two layers of heat bonded glass. A microfluidic channel, $100 \mu\text{m}$ wide and $40 \mu\text{m}$ deep, is etched in the top layer, and 200 nm thick Au electrodes with Ta adhesion are deposited in countersunk trenches in the bottom layer. Access to the microfluidic channel is provided via powder-blasted ports. The velocity and direction of the flow in the channel is controlled using a Fluigent MFCS-4C Microfluidic Flow Control System. The configuration of electrodes in the channel is shown in the inset of Fig. 1. Two sets of sensing electrodes, labeled as S_1 and S_2 , are connected to the microwave interferometer sensor and employed to detect the presence and altitude of cells in the channel. An actuation electrode, labeled as A, is used to actuate cells by a DEP force or apply PEFs to them (see Section II C).

We studied the dielectrophoresis response of single biological cells by measuring their vertical translation in the channel due to a DEP force. The DEP force acting on a cell is related to its dielectric properties through the Clausius-Mossotti factor, $K_{CM} = (\tilde{\epsilon}_c - \tilde{\epsilon}_m)/(\tilde{\epsilon}_c + 2\tilde{\epsilon}_m)$, with $\tilde{\epsilon}_c$ and $\tilde{\epsilon}_m$ the complex permittivity of the cell and suspension medium, respectively ($\tilde{\epsilon} = \epsilon - j\sigma/\omega$, with ϵ , σ , and ω the relative permittivity, conductivity, and angular frequency, respectively) and is given as⁶⁷

$$\vec{F}_{DEP} = \frac{3}{2} \epsilon_m V_c \text{Re}\{K_{CM}(\omega)\} \vec{\nabla} (E_{rms}^{DEP})^2. \quad (1)$$

Here, ϵ_m is the real part of the medium permittivity, V_c is the cell volume, and E_{rms}^{DEP} is the rms value of the electric field at the centre of the cell. $\text{Re}\{K_{CM}\}$ and subsequently the DEP force acting on a cell are functions of the cell structure and its inhomogeneous dielectric properties. The frequency dependent behavior of $\text{Re}\{K_{CM}\}$ for a viable CHO cell (using the double-shell dielectric model from Ref. 68) in a medium with conductivity 0.17 S/m is shown in Fig. 2. The effect of lowering the cytoplasm conductivity to that of the medium is also shown. Given that the cytoplasm conductivity dominantly affects the spectra over the frequency range of 1 – 30 MHz,⁶⁹ the DEP operating frequency was selected as 10 MHz to study the changes in the internal ionic content of cells.

The DEP force causes a vertical movement of cells in the channel. As shown in Fig. 3, a cell entering the channel from the left at an initial altitude h_1 is translated into altitude h_2 depending on the magnitude and direction of the DEP force acting on it in the actuation regions. The two sensing electrodes located on either side of the actuation electrode detect the

FIG. 2. $\text{Re}\{K_{CM}\}$ as a function of frequency for a CHO cell (parameters from Ref. 68) in an aqueous medium with the conductivity $\sigma_m = 0.17$ S/m. The solid curve shows the spectra for a typical normal CHO cell with $\sigma_{cyt} = 0.42$ S/m while the dashed curve is for a CHO cell with a lower cytoplasm conductivity, $\sigma_{cyt} = 0.17$ S/m.

FIG. 3. Schematic of the sensing (S_1 and S_2) and actuation (A) electrodes in the microfluidic channel and illustrative trajectory of a cell (not to scale) subjected to nDEP ($P_1 > P_2$, $\varphi < 0$), no DEP ($P_1 = P_2$, $\varphi = 0$), or pDEP ($P_1 < P_2$, $\varphi > 0$). The examples of measured sensing signals recorded for single CHO cells experiencing nDEP, no DEP, and pDEP are shown on the right. (The cell is shuttled back and forth in both directions. The trajectories are illustrated in only one direction.)

altitude of the cell before and after the DEP application. This is accomplished using a resonator-enhanced microwave interferometer to measure the capacitance change of sensing electrodes, S_1 and S_2 , due to a cell at microwave frequency (approximately 1.5 GHz). The details of the interferometer, shown in Fig. 1, and the sensing technique are explained elsewhere.^{70,71} The capacitance change in the sensing electrodes due to a cell is given as

$$\Delta C = 3\epsilon_m V_c \text{Re}\{K_{CM}|\omega_{RF}\}\left(\frac{|\bar{E}_{RF}|}{V_{RF}}\right)^2, \quad (2)$$

where V_{RF} is the voltage applied to the sensing electrodes and $|\bar{E}_{RF}|$ is the amplitude of the microwave electric field at the location of the cell. The corresponding signal from the interferometer is related to ΔC as $S(t) = G\Delta C(t)$ with G the gain of the interferometer. Since $|\bar{E}_{RF}|$ decreases with a distance from the electrodes, the capacitance change in the sensing electrodes is a function of the cell's altitude in the channel. Fig. 4 shows the peak value of ΔC as a function of the cell's altitude in the channel, obtained using Eq. (2).

The electronic signal measured by the interferometer as a cell traverses the channel (typically measured signals shown in Figs. 1 and 3) features two peaks, P_1 and P_2 , corresponding to the cell passing over each sensing electrode S_1 and S_2 , respectively. The amplitude of the peaks is proportional to the capacitance change in the electrodes and hence the cell's altitude in the channel (Fig. 4). DEP actuation causes a change in the altitude of the cell leading to P_2 larger (pDEP) or smaller (nDEP) than P_1 . We quantified the measured results using a parameter, force index, defined as $\varphi = (P_2 - P_1)/P_1$. The magnitude and sign of the force index for each cell is

FIG. 4. Capacitance change in the sensing electrodes as a function of a cell's altitude in the channel as obtained from Eq. (2).

related to the DEP force acting on it and, therefore, its dielectric properties (through $Re\{K_{CM}\}$, Eq. (1)).

C. Pulsed electric field exposure

The actuation electrodes employed for DEP application were also used to apply PEFs to cells. In our experiments, in order to study the temporal response of individual cells to PEFs, a single cell was selected and then continuously shuttled back and forth over the electrodes in the microfluidic channel (by periodical adjustment of inlet and outlet pressures). Fig. 5 shows the procedure used for a continuous DEP and EP application to a single cell and the simulated electric field profiles in the channel. We first measured the cell's DEP response at 10 MHz (sensitive to cell's internal conductivity) for approximately thirty to forty passes before electroporation. Then, as the cell passed once over the actuation region, we applied several 200 ns rise-time, 100 μ s duration trapezoidal pulses with the intensity in the range of 0.7–2.4 kV/cm to the cell. The repetition rate of pulses was set to 100 Hz or 200 Hz, and the number of applied pulses was dependent on the cell's velocity as it passed over the actuation electrodes. The total number of applied pulses was obtained by the time analysis of the recorded sensing and pulse generator signals. In order to generate pulses with the intensity in the range of 0.7–2.4 kV/cm, pulsed voltages of amplitude 5 V–15 V were applied to the actuation electrodes. Fig. 6 shows the simulation results of the intensity profile of the electric field in the actuation region for three different altitudes above the electrodes when a pulsed voltage with the amplitude of 10 V is applied to the actuation electrode. For a specific applied pulse amplitude, the actual intensity of the field applied to a cell is a function of its altitude in the channel and it is determined by post-processing analysis of the signal from the sensing electrodes. Immediately after electroporation, the DEP response of the cell was measured for several minutes as it continued moving back and forth in the channel.

D. Numerical method to determine cell's $Re\{K_{CM}\}$

The measured force indices for an individual cell is related to the DEP force acting on it and, hence, its $Re\{K_{CM}\}$ (Eq. (1)). Numerical simulations using *COMSOL Multiphysics 4.3b Particle Tracking for Fluid Flow* and *Electric Currents* modules were performed after obtaining

FIG. 5. Profiles of the electric field in the sensing and actuation regions of the microfluidic channel (panels on the left, vertical axis not to scale). (a) The sensing signal, S , (right) as the cell traverses the electrodes is proportional to the square of the amplitude of the sensing electric field (GHz). (b) The DEP force applied to a cell is proportional to the square of the amplitude of the DEP electric field (MHz). (c) The pulsed voltage applied to the actuation electrode (right) generates the PEF over two gap regions through which the cell traverses. (The cell is shuttled back and forth in both directions. The trajectories are illustrated in only one direction.)

FIG. 6. The intensity profile of the electroporation electric field in the actuation region for cells at altitudes 16 μm , 14 μm , and 12 μm when a pulsed voltage with the amplitude of 10 V is applied to the actuation electrode. Other field intensities are achieved by linearly scaling the applied voltage to the actuation electrode.

the experimental results to map the measured force indices for each cell (before and after electroporation) to its corresponding $Re\{K_{CM}\}$.

The laminar flow in the microfluidic channel has a parabolic velocity profile (Poiseuille flow) with zero value at the channel walls and maximum at the centre of the channel. With a mean flow velocity $\langle v_m \rangle$ and a channel height H , the fluid velocity, v_m , at any altitude from the bottom of the channel, h , is obtained from $v_m = 6\langle v_m \rangle \frac{h}{H} (1 - \frac{h}{H})$. The majority of cells sediment in the microfluidic channel to an equilibrium altitude before reaching the sensing and actuation electrodes. The equilibrium altitude is determined by equating the gravity, buoyancy, and hydrodynamic lift forces expressed by $\vec{F}_{grav-bouy} = \frac{4\pi}{3} r^3 g (\rho_c - \rho_m) (-\hat{y})$ and $\vec{F}_{lift} = C \frac{6\eta \langle v_m \rangle r^3}{H(h-r)} \text{sgn}(\frac{H}{2} - h) \hat{y}$, respectively. Here, g is the gravitational acceleration, ρ_c and ρ_m are the densities of the cell and suspension medium, r is the cell radius, η is the viscosity of the medium, h is the distance from the cell centre to the bottom of the channel, and C is a coefficient experimentally determined for our channel geometry as 0.031 in our previous study.⁶⁸ As the cell enters the DEP actuation region, its movement is governed by the DEP force given by Eq. (1) and the fluid drag force given by $\vec{F}_{drag} = 6\pi\eta r (\vec{v}_m - \vec{v}_c) \lambda$ with $\vec{v}_m$ and $\vec{v}_c$ the fluid and cell velocities, respectively, and λ is the ratio of the force experienced by a particle between two confining walls to the force in an unbounded fluid. λ is a function of the channel height and the cell's altitude in the channel and was calculated for our system from the data provided in the literature.⁷²

In the simulations, the channel, particle, and medium parameters were set according to our experiments and are listed in Table I. Using the developed numerical model, the cell's exit altitude (at the location of the second sensing electrode, S_2) was found for a range of $Re\{K_{CM}\}$

TABLE I. Channel, particle, and medium parameters employed in simulations.

Parameter	Symbol	Value	References
Channel height	H	40 μm	
Channel width	W	100 μm	
Cell density	ρ_c	1050 kg/m ³	73
Cell average radius	r	6–8 μm	Measured
Cell initial velocity	v_{c0}	1400–2000 $\mu\text{m/s}$	Measured
Medium density	ρ_m	1027 kg/m ³	Calculated
Medium viscosity	η	0.001 Pa s	74
Medium conductivity	σ_m	0.17 S/m	Measured
Medium permittivity	ϵ_m	78 ϵ_0	75

(within the range of -0.4 to 0.4). The capacitance change in the sensing electrodes was then calculated using Fig. 4, and the force index corresponding to each $Re\{K_{CM}\}$ was estimated by $\varphi = \frac{\Delta C|_{h_2} - \Delta C|_{h_1}}{\Delta C|_{h_1}}$. The cell's $Re\{K_{CM}\}$ was determined by matching the simulated force indices with the measured ones. The details of the numerical simulation procedure as well as an example plot of force index versus $Re\{K_{CM}\}$ are provided elsewhere.⁶⁸

E. Determination of cell's cytoplasm conductivity

With DEP actuation at 10 MHz, $Re\{K_{CM}\}$ is dominantly affected by the cell's internal conductivity. Employing the double-shell CHO dielectric model from Ref. 68, the percentage change in $Re\{K_{CM}\}$ at 10 MHz for 10% change in each cell dielectric parameter is provided in Table II. It is evident that the cytoplasm conductivity has the dominant effect on $Re\{K_{CM}\}$ at the selected DEP frequency. It should be noted that the membrane conductivity can be significantly affected by electroporation; however, its impact on $Re\{K_{CM}\}$ at 10 MHz is insignificant. The cell and nucleus size also have a significant effect on $Re\{K_{CM}\}$ at our measurement frequency (10 MHz). The change in the size of a cell's nucleus on the application of microsecond duration pulses has not been focused on in the previous studies, and therefore, is kept constant. However, the increase in the cell's size due to electroporation has been observed.^{51,76} Using the information obtained from the sensing signal, we incorporate its change into our simulations (the details are provided in Section III).

The cell's cytoplasm conductivity, σ_{cyt} , before and after electroporation, was determined using the double-shell dielectric model of CHO (parameters in Table II) and setting σ_{cyt} so that $Re\{K_{CM}\}$, calculated at 10 MHz, matched $Re\{K_{CM}\}$, obtained by the numerical model for the force index. In simulations, the nucleoplasm conductivity, σ_n , was chosen such that the ratio, $\sigma_n/\sigma_{\text{cyt}}$, was maintained at the value for untreated CHO cells, while other cell parameters remained at their nominal values (Table II). This is in accordance with the previous studies indicating that the nuclear envelope is capable of maintaining the ion concentration gradient between the nucleus and cytoplasm.^{46,77}

III. RESULTS AND DISCUSSION

A. DEP measurement of electroporated cells

Individual cells (with similar size, of spherical shape, and in the centre of the channel) were selected and then continuously shuttled back and forth in the microfluidic channel while

TABLE II. Relative change of $Re\{K_{CM}\}$ at 10 MHz for a ten percent change in each cell dielectric parameter. Dielectric model for a CHO cell from Ref. 68.

Parameter	Symbol	Nominal value	Change of $Re\{K_{CM}\}$ at 10 MHz (%) ^a
Membrane permittivity	ϵ_{mem}	$8.5 \epsilon_0$ F/m	0.3
Membrane conductivity	σ_{mem}	1×10^{-6} S/m	0.0002
Cytoplasm permittivity	ϵ_{cyt}	$60 \epsilon_0$ F/m	0.005
Cytoplasm conductivity	σ_{cyt}	0.42 S/m	12
Nuclear envelope permittivity	$\epsilon_{n-\text{env}}$	$23.2 \epsilon_0$ F/m	1.65
Nuclear envelope conductivity	$\sigma_{n-\text{env}}$	1×10^{-3} S/m	0.058
Nucleoplasm permittivity	ϵ_n	$120 \epsilon_0$ F/m	0.028
Nucleoplasm conductivity	σ_n	1.50 S/m	0.89
Nuclear envelope thickness	$d_{n-\text{env}}$	40 nm	1.24
Nucleus radius	R_n	3.3 nm	4.6
Membrane thickness	d_{mem}	5 nm	0.22
Cell radius	R_{cell}	6 nm	3.2

^aMedium conductivity, σ_m , and permittivity, ϵ_m , are set to 0.17 S/m and $78 \epsilon_0$ F/m, respectively.

their DEP response was measured. Their response was measured before and after exposure to $100\text{ }\mu\text{s}$ duration pulsed electric fields (PEFs) with the intensity ranging from 0.7 to 2.4 kV/cm . Six to eight pulses (depending on the cell velocity) with a repetition rate of 100 Hz was applied to each cell. For two cases, an intensity of 2.2 kV/cm and 15 pulses with a repetition rate of 200 Hz was employed. For each cell, the DEP signal was recorded for every pass over the electrodes (typically 100 passes for a 5-min span). At first, no DEP was applied to the cell, which resulted in force indices close to zero (Fig. 7(b)). Then, a 10 MHz and 7 V_{pp} sinusoidal DEP voltage was applied to the actuation electrodes to measure the cell's force index before the electroporation. The cell's DEP response before the pulse exposure was measured for approximately 1 minute. Then, a multiple pulse voltage with the desired amplitude was applied to the actuation electrodes, and the cell experienced the PEFs as it once passed over the actuation regions. The amplitude of the applied electric field was obtained from the simulation of the

FIG. 7. Example of measured results for a single CHO cell shuttled back and forth over the electrode array in the microfluidic channel with $\sigma_m = 0.17\text{ S/m}$ and subjected to seven $100\text{ }\mu\text{s}$ pulses with the amplitude of 1.5 kV/cm and a repetition rate of 100 Hz . (a) Sensing signals registered for four passes of the cell over the sensing electrodes (10 s interval right at the time of pulse application). The first signal, labeled as 1, is for the cell actuated by a DEP force just before electroporation. The second signal, labeled as 2, is during the period when the pulses are applied (during this period, pulsed electric voltages are applied to the actuation electrodes). The third and fourth signals, labeled as 3 and 4, are for the cell actuated by a DEP force after electroporation. These sensing signals recorded over approximately 5-min period are used to obtain the force indices, peak amplitudes, and velocity information plotted in (b), (c), and (d). (b) Measured force indices before and after electroporation. 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 the DEP application (at 10 MHz), the cell experiences a strong pDEP force. After electroporation (shown by a solid black line), the intensity of the pDEP force decreases. (c) Amplitude of the peak, P_1 , recorded by the first sensing electrode pair that the cell passes over. (d) Velocity of the cell as it moves back and forth in the channel. Fluctuations in the velocity cause slight changes in the cell's altitude and amplitude of P_1 . P_1 and velocity are used to estimate the average altitude of the cell in the channel before and after electroporation (required for post-processing simulations).

field intensity in the channel after finding the altitude of the cell during the pulse exposure pass. After pulse application, the actuation electrode voltage was set back to a 10 MHz and 7 V_{pp} sinusoidal signal, and the cell's force index after electroporation was measured. The first measurement was a few seconds after the pulse exposure (the time required for the cell to return in the channel and reach the electrodes).

Figure 7 shows an example measurement result for a cell subjected to seven pulses with the amplitude of 1.5 kV/cm over a time period of 5 minutes. An example of recorded sensing signals as the cell is cycled over the electrodes right before electroporation, during the pulse application, and after electroporation is presented in Fig. 7(a) (a 10 s interval right at the time of pulse application). These sensing signals recorded over a 5-min period are used to obtain the force indices, peak amplitudes, and velocity information presented in Figs. 7(b)–7(d). Each bar in Fig. 7(b) corresponds to the force index for one pass of the cell over the electrodes. Red lines represent the average of force indices with error bars being the standard deviation. With no DEP application (initial 30 s), the average force index is close to zero. During the DEP application (next 100 s), the cell experiences a pDEP force resulting in positive force indices. After the pulse exposure, the cell still experiences a pDEP force; however, the force index significantly decreases within several seconds after the pulse application. There are fluctuations in the measured force indices since the cells are not perfectly spherical and they rotate as they move back and forth, they have an inhomogeneous and asymmetric internal structure, and there is a slight variation in the altitude and velocity of the cell over time. However, the measured change in the average force indices before and after electroporation is considerably larger than the fluctuations. The amplitude of P_1 and the cell's velocity over the measurement period are plotted in Figs. 7(c) and 7(d), respectively. We used these to obtain the cell's altitude information required for post-processing simulations.

Measurements were performed on more than 20 individual cells subjected to PEFs with the amplitude ranging from 0.7 to 2.4 kV/cm. The dynamic response of the normalized force indices for five of these cells, subjected to pulses with amplitudes 0.7, 1.2, 1.5, 1.8, and 2.2 kV/cm, is shown in Fig. 8 (4-point moving average employed on the measured data similar to Fig. 7(b) for each cell). For pulse amplitudes 0.7–1.8 kV/cm, the cells were subjected to six or seven pulses with a repetition rate of 100 Hz. For the pulse amplitude 2.2 kV/cm, the cell was subjected to fifteen pulses with a repetition rate of 200 Hz. A comparable behavior was observed

FIG. 8. Normalized force indices (4-point moving average) for four cells subjected to six or seven 100 μ s duration pulses with the amplitudes 0.7, 1.2, 1.5, and 1.8 kV/cm and the repetition rate of 100 Hz and one cell subjected to fifteen 100 μ s duration pulses with the amplitude of 2.2 kV/cm and the repetition rate of 200 Hz. Solid lines represent an exponential fit to the measured data points. The dashed line indicates when the electroporation pulse is applied. Medium conductivity is 0.17 S/m.

for other measured cells. It was noted that while electric fields above 1.2 kV/cm caused a measurable change in the force index (and dielectric properties) of the cells, a 0.7 kV/cm field intensity was not sufficiently strong to affect the cells. This is consistent with the findings in other reports^{34,43,50,78–81} where similar pulses were employed and optical techniques were used to measure the permeability of dyes into cells due to electroporation.

Cell swelling has been associated with the conventional electroporation (pulses in millisecond and microsecond duration range) in several previous studies.^{51,76} While Zhuang *et al.* reported no significant change in the size of Jurkat cells after electroporation with 8, 100 μ s, and 1.1 kV/cm pulses.⁴⁷ Moisesescu *et al.* observed a thirty percent increase in the diameter of murine melanoma cells after the exposure to 8, 100 μ s, and 1.8 kV/cm pulses.⁵¹ Golzio *et al.* also reported an increase in the diameter of CHO cells by thirty percent caused by 5 ms and 0.8 kV/cm pulses.⁷⁶ In our experiments, an expansion in the size of cells was observed for PEFs with the amplitudes greater than 1.8 kV/cm, and we considered it in our simulations. This observation was based on a noticeable increase in the amplitude of P_1 immediately after electroporation (note that the cell altitude did not change significantly, as verified by monitoring the cell velocity, Fig. 7(d)).

To verify that the applied electroporation pulses did not cause thermal effects, simulations were performed using *COMSOL Multiphysics Heat Transfer* module. For a 100 μ s duration, trapezoidal pulse with the intensity of 2.4 kV/cm (maximum intensity employed in experiments), and 200 ns rise-time, the maximum temperature increase in the channel during the pulse was 0.3 K. In the simulation, the temperature decayed back to almost its initial value after 10 ms (for a repetition rate of 100 Hz, the pulses are applied every 10 ms), assuring a negligible average temperature increase for repetitive pulse applications.

The temporal response of the cells after electroporation (examples are presented in Fig. 8) indicates there is an observable time constant associated with dielectric change, ranging from 3 to 30 s depending on the applied pulse intensity. At the measured frequency, 10 MHz, this is primarily due to the changes in the cytoplasm conductivity. The dielectric change in the cells exposed to stronger pulses occur faster due to the higher density of created pores as well as the larger drift current through the pores. The resealing time of pores reported in the literature ranges from milliseconds to minutes,^{31,80,82–85} and there are studies that suggest several phases with various time constants for pore resealing and membrane recovery.^{31,80,83,85} Our observation demonstrates the existence of ion permeable pores in the membrane tens of seconds after the pulse application. This agrees with the results published by Son *et al.*⁸⁶ based on numerical modeling of electroporation, which showed that the transport of Calcium ions into a cell continues up to 10 s after the application of a single 100 μ s duration pulse with the amplitude of 1 kV/cm. In their simulations, the lifetime of pores was assumed to be 4 s; however, after resealing the pores remained (at their minimum size ~ 1 nm) for a longer time (more than 20 s).⁸⁶ Using Thallium ions with fluorescent microscopy (preloading cells with a dye which fluoresces upon entry of Thallium cations into cells after electroporation), Bowman *et al.* have reported a lifetime in the range of 10 min for nanopores created by nanosecond duration pulses.⁸⁷

B. Cytoplasm conductivity analysis

The cytoplasm conductivity, σ_{cyt} , of each cell before and after electroporation in a medium with the conductivity $\sigma_m = 0.17$ S/m was obtained by the numerical simulation (explained in Sections IID and IIE) and is shown in Fig. 9. Square and circle data points show cytoplasm conductivities before and after the PEF application, respectively. Solid markers represent cells subjected to 6–8 pulses with a repetition rate of 100 Hz, and the open ones represent the cells subjected to fifteen pulses with a repetition rate of 200 Hz. The error bars indicate the confidence interval for the estimated cytoplasm conductivity of each cell as obtained by considering the standard deviation of their measured force indices (example represented in Fig. 7(b) for one cell). The cytoplasm conductivity of cells may vary before pulse applications due to the variability in the state of cells. In all cases, cytoplasm conductivity consistently decreased after

FIG. 9. Cytoplasm conductivity of single CHO cells before (square) and after (circle) exposure to $100\ \mu\text{s}$ duration pulses with various intensities in a medium with $\sigma_m = 0.17\ \text{S/m}$. Solid markers represent the results for six to eight pulses with a repetition rate of 100 Hz, and open markers show the results for fifteen pulses with a repetition rate of 200 Hz.

electroporation and pulses with higher intensities caused a larger decrease in the cell's internal conductivity. Given that the ion concentration in the suspension medium ($\sigma_e = 0.17\ \text{S/m}$) is less than that in the cell's cytoplasm, electrodiffusion during pulse application and diffusion during the pore resealing period result in a large efflux of ions (predominantly potassium, sodium, and chloride) from the cell. This happens to a larger extent with stronger pulses since they create a larger number of pores in the cell membrane (providing more pathways for ion transport) and cause a larger drift induced current. It should be noted that looking at the dielectric properties of individual cells rather than bulk measurement makes it possible to observe substantial changes in each cell even with lower pulse amplitudes (less than $1.8\ \text{kV/cm}$). Averaging over a population of cells may understate the effect of pulses due to variability in the initial condition of cells.

In our experiments, the cell's internal conductivity after electroporation never decreased to lower than approximately $0.15\ \text{S/m}$. For pulses with the intensities $>2\ \text{kV/cm}$, increasing the number of pulses to fifteen (open marker data points in Fig. 9) or applying two sets of fifteen pulses with a 3 s interval (data not shown) did not lead to a further decrease in the cytoplasm conductivity. Although the observed threshold for a decrease in the cell's cytoplasm conductivity, approximately $0.15\ \text{S/m}$, is close to the external medium conductivity, $0.17\ \text{S/m}$, it does not imply the equilibrium of ion concentrations inside and outside the cell as one might expect upon creation of large pores in the membrane. The mobility of ions inside the cell is significantly lower than the suspension medium (by a factor of 3–4).^{88,89} Thus, an internal ion concentration in equilibrium with the external medium would result in a cytoplasm conductivity approximately $0.05\ \text{S/m}$, which was not observed. One explanation for the threshold is the presence of large and relatively immobile negative ions inside the cell, which attract mobile positive ions (mainly sodium and potassium) to maintain cell electrical neutrality.^{90,91} A decrease in the cytoplasm conductivity to $0.1\ \text{S/m}$ has been reported in another study,⁴⁶ where the dielectric parameters of a population of electroporated Jurkat cells (in a medium with conductivity $0.1\ \text{S/m}$) were measured using a time-domain dielectric spectroscopy technique. Although the result is for nanosecond duration pulse application, it is consistent with the finding here.

To examine whether the $0.15\ \text{S/m}$ threshold for the cell's cytoplasm conductivity after electroporation is independent of the conductivity of the external medium, the electroporation of several individual cells suspended in a medium with the conductivity $\sigma_m = 0.05\ \text{S/m}$ was studied. The DEP response of single cells was measured before and after electroporation using eight $100\ \mu\text{s}$ duration pulses with the intensity of $2.2\ \text{kV/cm}$. If the cytoplasm conductivity decreases to that of the medium, $\sigma_{\text{cyt}} \leq 0.05\ \text{S/m}$, this would result in $\text{Re}\{K_{CM}\} \leq 0$ and hence a zero or negative DEP force acting on the cell (zero or negative force index). Our results showed that the force index of all the measured cells decreased substantially after electroporation, but remained consistently positive ($\text{Re}\{K_{CM}\} > 0$), implying that a set concentration of ions is

always maintained inside the cell. The average cytoplasm conductivity after electroporation (of all measured cells) was approximately 0.13 S/m. This is close to the result obtained for the cells in a medium with conductivity 0.17 S/m.

IV. CONCLUSIONS

In this paper, quantitative results on the change in the cytoplasm conductivity of single CHO cells due to electroporation with pulsed electric fields of 100 μ s duration and various intensities were presented. Using a microfluidic system, we simultaneously electroporated and measured the DEP response of individual CHO cells. The results showed that, with the electroporation in a low conductivity medium, the internal conductivity of cells decreased after electroporation depending on the intensity of the applied pulses. We observed that, for cells exposed to strong pulsed electric fields, the cytoplasm conductivity did not decrease below a threshold. We hypothesize this is due to the presence of large and relatively immobile negative ions inside the cell, which attract mobile positive ions to maintain cell electrical neutrality. By monitoring the temporal DEP response of cells after electroporation, we observed there was a time constant associated with the induced dielectric changes (primarily the cytoplasm conductivity). This ranged from seconds to tens of seconds depending on the intensity of applied pulses. These results support recent numerical simulations reported in the literature.⁸⁶ The presented result can be used to infer the information about the density and resealing time of very small pores and their relationship with the pulse intensity.

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