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Gene delivery by electroporation after dielectrophoretic positioning of cells in a non-uniform electric field

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Abstract

We report the use of dielectrophoresis (DEP) to position U-937 monocytes within a non-uniform electric field, prior to electroporation (EP) for gene delivery. DEP positioning and EP pulsing were both accomplished using a common set of inert planar electrodes, micro-fabricated on a glass substrate. A single-shell model of the cell's dielectric properties and finite-element modeling of the electric field distribution permitted us to predict the major features of cell positioning. The extent to which electric pulses increased the permeability of the cell membranes to florescent molecules and to pEGFPLuc DNA plasmids were found to depend on prior positioning. For a given set of pulse parameters, EP was either irreversible (resulting in cytolysis), reversible (leading to gene delivery), or not detectable, depending on where cells were positioned. Our results clearly demonstrate that position-dependent EP of cells in a non-uniform electric field can be controlled by DEP.

Keywords: Electroporation; Electropermeabilization; Dielectrophoresis; Non-uniform electric field; Gene delivery; Transfection; Micro-electrode

1. Introduction

Biological cells of various types can be distinguished from one-another and displaced within a liquid medium using dielectrophoresis (DEP) [1]. In a spatially non-uniform electric field, E, the differential electric polarizability of cells and their suspending medium produces the DEP force, which can be either attractive (towards the strong-E regions) or repulsive (towards the weak-E regions), depending on experimental conditions. Attractive or repulsive DEP forces are usually referred to as "positive" (pDEP) or "negative" (nDEP), respectively. Measurement of the DEP force as a function of experimental variables produces DEP "spectra" which are characteristic of each cell-type, allowing for cell separation and identification [2,3]. In microfluidic devices, DEP has been used to transport and position cells with sufficient precision to enable single-cell manipulation [4–6].

Microfluidic devices for single-cell or sub-cellular analysis often use electric field-based techniques other than DEP to increase permeability of the cell membrane [7–9], or to induce cytolysis [10,11]: Electroporation or electropermeabilization (EP) results from the application of an intense electric field to bring about structural changes of the cell membrane that increase its permeability. It is well known that irreversible EP leads to cytolysis [12], while reversible EP can be used to transfer molecules such as DNA into the cells while maintaining high rates of cell survival [13]. Generally, pulsed electric fields are used and the extent of EP is determined by parameters such as the strength, duration, and repetition rate of the electric pulses. Critical values of the electric field strength, which determine whether cell membrane EP is reversible or irreversible, are specific to each cell-type and are usually determined by performing experiments at different E values [14,15].

Traditionally, *in vitro* EP has been accomplished using electrodes with millimeter spacing, and the position of individual

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cells within the electrode chamber did not need to be considered [16]. In contrast, micro-fabricated devices for EP accommodate relatively small numbers of cells and their smaller (sub-mm) electrode dimensions require consideration of spatial non-uniformities in E and of cell positioning with respect to the electrodes [7–11]. Cell positioning by DEP is known to complement EP experiments. For example, the alignment of cells by DEP after EP has been used for cell–cell fusion [17], and EP of DEP-trapped cells increased the sensitivity of impedance-based cell-detection [18]. In a spatially non-uniform E, the dependence of EP on the field's amplitude results in position-dependent EP, and therefore leads to regions within the chamber where either reversible or irreversible EP may prevail [19,20].

In the present report, we have used spatially non-uniform E to assess the extent of reversible and irreversible EP as a function of cell position within the electrode chamber. As a first step, position-dependent EP of a single cell-type, U-937 [21], was investigated using florescent probes. We selected a single cell-type to ensure that pulsing conditions required for EP were similar for all cells, such that any differences in EP would primarily be due to their positioning. We then used DEP to accentuate position-dependent EP, by moving cells into specific regions within the electrode chamber. Gene delivery was accomplished for all cases of cells being randomly distributed, or selectively positioned by DEP prior to EP, however the number of successfully transfected cells and their viability depended on the specific conditions of EP and of DEP.

2. Methodologies

2.1. DEP analyses using a single-shell dielectric model of a spherical cell

Dielectric parameters were determined independently of EP experiments, by fitting the measured cross-over frequency from DEP experiments (defined in Eq. (3) below) with a single-shell model of the spherical cell [22,23]. Different values of the complex electrical permittivity, ε^*, were assigned to the external medium, $\varepsilon_e^*=\varepsilon_e-j\sigma_e/\omega$, to the cell membrane, $\varepsilon_m^*=\varepsilon_m-j\sigma_m/\omega$, and to the (internal) cytosol, $\varepsilon_i^*=\varepsilon_i-j\sigma_i/\omega$, where ε designates permittivity, σ electrical conductivity, and $\omega=2\pi f$ angular frequency, f being the frequency of the applied sinusoidal electric field, and $j=\sqrt{-1}$. The *relative* permittivity is $\kappa=\varepsilon/\varepsilon_0$, ε_0 being the permittivity of free space.

The DEP phenomena observed in the present work can be modeled using the following four equations (Eqs. (1)–(4)) [25]: The DEP force, F_{DEP}, for the case of a spherical cell of radius, a, is approximated by

$$F_{DEP}=2\pi\varepsilon_e a^3 Re[K(\omega)]\nabla E^2, \tag{1}$$

where the polarization factor, K, is

$$K(\omega)=\frac{\varepsilon_{cell}^*-\varepsilon_e^*}{n\varepsilon_{cell}^*+(n+1)\varepsilon_e^*}, \tag{2}$$

where $\varepsilon_{cell}^*=\varepsilon_{cell}-j\sigma_{cell}/\omega$ is the complex electrical permittivity of the cell and n is the multipolar term (for the present spherical case, we assumed a pure dipole, $n=1$). nDEP and pDEP correspond to $K<0$ and $K>0$, respectively. A single "cross-over" frequency, f_0, defined by $K(f_0)=0$ and $F_{DEP}(f_0)=0$, was seen in DEP experiments (described below) when $f<10^7$ Hz:

$$f_0=\frac{1}{2\pi}\left(\frac{(\sigma_e-\sigma_{cell})(\sigma_{cell}+2\sigma_e)}{(\varepsilon_{cell}-\varepsilon_e)(\varepsilon_{cell}+2\varepsilon_e)}\right)^{\frac{1}{2}} \tag{3}$$

The effective complex permittivity of the cell, ε_{cell}^*, based on the above single-shell theory is

$$\varepsilon_{cell}^*=\varepsilon_m^*\frac{\left(\frac{a}{a-d}\right)^3+2\frac{\varepsilon_i^*-\varepsilon_m^*}{\varepsilon_i^*+2\varepsilon_m^*}}{\left(\frac{a}{a-d}\right)^3-\frac{\varepsilon_i^*-\varepsilon_m^*}{\varepsilon_i^*+2\varepsilon_m^*}}, \tag{4}$$

where d is the membrane's thickness. This model has been used in previous work to measure ε_m^*, ε_i^* and the area-specific conductance of the membrane, $G_m=\sigma_m/d$, for several cell-types, using DEP [2,3,22,23] and the electrorotation technique [24,26,27]. In our study we took fixed values for a, d, σ_m, ε_i and ε_e. We assumed $a=7.5$ μm (from optical measurements), $d=7$ nm [27], $\sigma_m=10^{-6}$ S m^{-1}, and $\varepsilon_i=\varepsilon_e=80\varepsilon_0$, as will be further discussed below. The experimental conditions determined f and σ_e, that were in the ranges $10^4<f(\text{Hz})<10^7$ and $10^{-3}<\sigma_e$ (S m^{-1}) <1.6. The remaining two parameters, ε_m and σ_i, were then found by fitting experimental f_0 data using Eq. (2) ($K(f_0)=0$), with ε_{cell}^* given by Eq. (4) and restricting the fit parameters to the ranges $0.2<\sigma_i$ (S m^{-1}) <1; and $3\varepsilon_0<\varepsilon_m<23\varepsilon_0$. The differences between measured and calculated values of f_0 were minimized using a least-squares algorithm (lsqcurvefit, Matlab v. 7.2, The Mathworks, Natick, MA).

The polarization factor $K(f,\sigma_e)$ determines cell positioning by DEP, where nDEP occurs when K(low-f) <0 and pDEP when K (high-f) >0. Using parameters found from best fits presented below in the results ($\varepsilon_m=6.0\varepsilon_0$, $\sigma_i=0.425$ S m^{-1}), the cross-over frequency, f_0, is seen to increase when σ_e increases (from A to B to C to D in Fig. 1). When $\sigma_e>>\sigma_i$, $K<0$ for all values of f, and only nDEP can occur (Fig. 1, curves C and D).

2.2. Fabrication of electrodes and modeling of the electric field

Planar Ti/Pt electrodes were fabricated on glass substrates using standard lift-off processes [28]: Chromium masks were

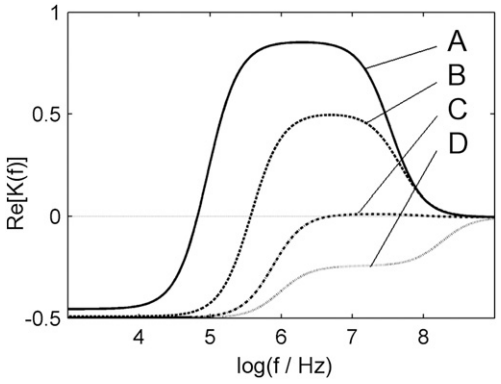

Fig. 1. The real part of the polarization factor, $Re[K(f)]$, in Eqs. (1) and (2) versus frequency, f, for model parameters corresponding to U-937 monocytes and at different values of σ_e. A: $\sigma_e=0.0175$ S m^{-1}; B: $\sigma_e=0.1$ S m^{-1}; C: $\sigma_e=0.4$ S m^{-1}; and D: $\sigma_e=1.0$ S m^{-1}.