Dielectrophoresis (DEP) of Spherical Cells after Nano-Second Pulse Electric Field (nsPEF) Induced Electroporation

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1. Abstract

In this study, theoretical models are used to predict the dielectrophoretic (DEP) force on spherical cells. The dielectrophoretic responses of both normal and malignant cells are used to determine frequency ranges, medium permittivity and conductivity that favor intact separation of cells using the DEP force. Normal and malignant Tonsillar B-cells are used in this study.

2. Introduction

In recent years, research has focused on separation of malignant cells from normal cells\cite{1,2,3,4}. After the destruction of malignant cells, a method must be developed to successfully separate malignant from the normal cells by changing their relative location and alignment. Dielectrophoresis (DEP) is an effective method for separation of cells and other micro substances. Dielectrophoresis is not a new phenomenon; every dielectric material experiences a force when a non-uniform electric field is applied to it. The electric field creates a force that acts on the induced dipoles in a direction that faces either the high electric field region (positive DEP) or low electric field region (negative DEP). Dielectrophoresis is used in biological applications like levitation, single cell trapping, cellular orientation in pearl-like chains for electrofusion, and separation of normal from malignant cells. DEP response depends on the frequency-dependent permittivity of spherical cells and medium (water-like), size of cells and overall morphology. Study has been done on the dielectrophoretic response of intact membranes; however, in this study the dielectrophoretic response of electroporated spherical cells is investigated.

High-intensity, nanosecond, pulsed electric fields (nsPEFs) may be used for piercing and creating pores in cell membranes\cite{1}. nsPEFs is a non-thermal tool that both affects cell membranes and reaches the interior of cellular organelles. nsPEFs have various effects on
spherical cells (like human cells) depending on the magnitude and frequency of the pulse. Recently, nsPEFs have been used in numerous applications like killing of tumor cells\cite{5}, temporary blockage of action potential propagation in nerves\cite{6}, cellular electroporation\cite{2}, electrically triggered intracellular calcium release\cite{7}, nonthermal destruction of microorganisms\cite{2}, and disruption of voltage-gated channels\cite{8}. In this paper, we focus on DEP and its effectiveness in separating nsPEF electroporated malignant cells from normal cells.

At a particular frequency and magnitude, nsPEF could be used to create pores or holes through the cells (electroporation), which makes it a potential method for restrictive drug delivery. The dielectric properties of electroporated spheroidal cells are different from that of normal cells which also makes their dielectrophoretic behavior different. To investigate DEP after nsPEF induced electroporation of the spherical cells, the real part of the complex Clausius-Mossotti function, which includes the complex permittivity of the medium and the spherical cells, along with the magnitude of the electric field is used to determine the frequency dependent DEP force.

3. Dielectrophoresis

Fig. 1 (a) Before DEP; polarization align (Red, positive DEP) or oppose the direction of the varying electric field (Blue, negative DEP). (b) Separation of cells after DEP.

The varying electric field polarizes the cell which causes either a positive or negative DEP force. The DEP force depends on the volume and effective complex permittivity of the cell, the electric field gradient, and the real part of the Clausius-Mossotti (CM) factor by:
\[ F_{\text{DEP}} = \frac{3}{2} v_{\text{cell}} \epsilon_{\text{med}} \text{Re}[K_{\text{CM}}^*(f)] \nabla E^2 \]  

(1.1)

\[ K_{\text{CM}}^*(f) = \frac{\epsilon_{\text{cell}}^* - \epsilon_{\text{med}}^*}{\epsilon_{\text{cell}}^* + 2 \epsilon_{\text{med}}^*}, \quad \epsilon_i^* = \epsilon_i - j \sigma_i / \epsilon_0 \omega \]  

(1.2)

where \( F_{\text{DEP}} \) is the DEP force, \( v_{\text{cell}} \) is the volume of the cell, \( K_{\text{CM}}^*(f) \) is the CM factor, \( \nabla E^2 \) is the square of the electric field gradient, \( \epsilon_{\text{cell,med}}^* \) is the complex permittivity of the cell and medium respectively, \( \sigma_i \) is the conductivity, and \( \omega \) is the angular frequency.

4. Dielectrophoretic Effects

The effective complex permittivities of the spherical cells are obtained by successfully utilizing the single-shell model result. In this process, the nuclear cytoplasm and the inner membrane surrounding it are replaced by a single, homogeneous core. The new core is surrounded by cytoplasm, which leads to the repetition of the calculation involving the effective permittivity of the new core \( \epsilon_{\text{eff1}}^* \) and the permittivity of the cytoplasm \( \epsilon_c^* \). The effective permittivity of the whole cell is determined by the same equation using \( \epsilon_{\text{eff2}}^* \) and \( \epsilon_m^* \) as:

\[ \epsilon_{\text{eff1}}^* = \epsilon_{\text{om}}^* \frac{3 \epsilon_{\text{om}}^* + (\epsilon_{oc}^* - \epsilon_{\text{om}}^*)(1 + 2V_3)}{3 \epsilon_{\text{om}}^* + (\epsilon_{oc}^* - \epsilon_{\text{om}}^*)(1 - V_3)} \]  

(2.1)

\[ \epsilon_{\text{eff2}}^* = \epsilon_c^* \frac{3 \epsilon_c^* + (\epsilon_{\text{eff1}}^* - \epsilon_c^*)(1 + 2V_2)}{3 \epsilon_c^* + (\epsilon_{\text{eff1}}^* - \epsilon_c^*)(1 - V_2)} \]  

(2.2)

\[ \epsilon_{\text{cell}}^* = \epsilon_m^* \frac{3 \epsilon_m^* + (\epsilon_{\text{eff2}}^* - \epsilon_m^*)(1 + 2V_1)}{3 \epsilon_m^* + (\epsilon_{\text{eff2}}^* - \epsilon_m^*)(1 - V_1)} \]  

(2.3)

The parameters for the various compartments of the cell are displayed in Table I.
Table I. Parameters of normal and malignant (Farage) Tonsillar B-cells.

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conductivities (S/m):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Environment</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Cell Membrane</td>
<td>5.6 x 10^{-5}</td>
<td>9.1 x 10^{-6}</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>1.31</td>
<td>0.48</td>
</tr>
<tr>
<td>Nuclear Envelope</td>
<td>1.11 x 10^{-2}</td>
<td>4.4 x 10^{-3}</td>
</tr>
<tr>
<td>Nucleoplasm</td>
<td>2.04</td>
<td>1.07</td>
</tr>
<tr>
<td><strong>Dielectric Constant:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Environment</td>
<td>80.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Cell Membrane</td>
<td>12.8</td>
<td>7.0</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>60.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Nuclear Envelope</td>
<td>106</td>
<td>60.3</td>
</tr>
<tr>
<td>Nucleoplasm</td>
<td>120.0</td>
<td>120.0</td>
</tr>
<tr>
<td><strong>Geometry (μm):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radius of cell</td>
<td>3.3</td>
<td>5.12</td>
</tr>
<tr>
<td>Thickness of cell membrane</td>
<td>7 x 10^{-3}</td>
<td>7 x 10^{-3}</td>
</tr>
<tr>
<td>Radius of nucleus</td>
<td>2.8</td>
<td>4.4</td>
</tr>
<tr>
<td>Nuclear Envelope</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

5. Discussion of Simulations and Results

Various simulations were done to determine the frequency dependent DEP force. In this simulation the permittivity was modeled with a constant real value. Fig. 2 shows the real effective permittivity of the spherical cell as a function of input frequency for a range of 10^{4} Hz to 10^{10} Hz. The significant change in real effective permittivity occurs at a frequency range of 10^{6} Hz to 10^{7} Hz.
Using the same constant real permittivity model, a simulation was done to determine the DEP force on the normal and malignant cells. This was carried out by observing the real part of the CM factor. The DEP force is directly proportional to the real part of the CM factor as shown in equation (1.1). This means that the DEP force and CM factor at particular frequencies are similar but vary only in magnitude and unit representation. As a part of this simulation, the relative permittivity of the medium was changed from 80 to 5 and the effect on the CM factor was observed at a constant conductivity of 0.6 S/m. Next the conductivity was changed from 0.6 S/m to 0.05 S/m and the corresponding effects on the CM factor were observed at constant medium permittivity as shown in Fig. 3.
Fig. 3 Normal and Malignant CM factors for a frequency range of $10^4$ Hz to $10^{10}$ Hz, $\varepsilon_{r,med} = 80$ and $\sigma = 0.55$ S/m, 0.05 S/m.

Table II. Parameters for epsilon for the Debye relaxation model.

<table>
<thead>
<tr>
<th>Cell compartments</th>
<th>$\varepsilon_s$</th>
<th>$\varepsilon_\infty$</th>
<th>$f_{relax}$ (MHz)</th>
<th>$\sigma_{dc}$ (S/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane</td>
<td>11.70</td>
<td>4.00</td>
<td>179.85</td>
<td>1.10 x $10^{-7}$</td>
</tr>
<tr>
<td>Bound Water</td>
<td>34.65</td>
<td>5.46</td>
<td>1760</td>
<td>0.66</td>
</tr>
<tr>
<td>Extra-cell, and cytoplasm</td>
<td>67.00</td>
<td>5.00</td>
<td>17900</td>
<td>0.55</td>
</tr>
</tbody>
</table>

In another simulation, the effect of a varying real permittivity was observed. The model used for this simulation is the normal Debye relaxation model:

$$
\varepsilon^*(f) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + j \frac{f}{f_{relax}}} + \frac{\sigma_{dc}}{j\varepsilon_0 2\pi f}
$$

(3.1)

and the parameters used for this part are shown in Table II.
Fig. 4 Effects of a frequency dependent real permittivity of cell on (a) CM factor for $\varepsilon_r = 67$ and $\sigma = 0.55$ S/m (b) $\varepsilon_r = 67$ and $\sigma = 0.05$ S/m

In the final simulation, the effect of electroporation is observed. Electroporation increases the conductivity of cell membranes by an order of 4 for low intensity pulse and an order of 6 for
high intensity pulse approximately. Fig. 5 shows the CM factor of cells after nsPEF induced electroporation for no pulse, low intensity and high intensity pulses.

![Graph showing CM factor vs Frequency](image)

Fig. 5 Effects of electroporation by low intensity and high intensity pulse on the CM factor of cells.

6. **Relevance to Engineering Education**

   This undergraduate project provides a research environment in which the student uses mathematical simulations to predict real life results based on knowledge of the subject. It is important to note that real life results are not exactly the same as in the simulations since various external factors would mildly limit the accuracy of mathematical models. As a result, the student learns through this process that equations can only predict the approximate behavior of physical systems and in this case provide a basis for future experiment that would separate cancer cells from healthy cells.

7. **Summary and Conclusion**

   The simulations show the frequency dependence of effective permittivity of normal and malignant cells, and the corresponding CM factor for the cells which directly correlates with the DEP force on the cell when a varying electric field is applied. The frequency range at which DEP
can be used to separate the normal cells from the malignant is dependent on the permittivity model, values and on the conductivity of the medium. Also by using different models for characterizing the real part of the permittivity of the cells, the magnitude and frequency span of the significant change in the CM factor varied. The best behavior for separation of cells using DEP was found in the medium with relative permittivity of 80 and conductivity of 0.6 S/m. It was also shown that electroporation affects the CM factor significantly with high intensity pulse. Separation of electroporated cells is more probable with a high intensity pulse.

Bibliography