Supplementary Information

Low frequency stimulation induces polarization-based capturing between normal, cancerous and white blood cells; A new separation method for circulating tumor cells enrichment or phenotypic cell sorting

Authors

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Laminarity verification by Reynolds Number (Re):

Laminarity in flow is measured by the Reynolds number (Re): [1][2]

\[ Re < 2300 \text{ (laminar)} \]
\[ 2300 < Re < 2900 \text{ (transient)} \]
\[ 2900 < Re \text{ (turbulent)} \]

\[ Re = \frac{\rho v L}{\mu} \]

\( \rho \): fluid density \( \frac{kg}{m^3} \),
\( v \): fluid speed \( \frac{m}{s} \),
\( L \): Restrictive Length/ tube diameter (m),
\( \mu \): dynamic viscosity \( \frac{kg}{m.s} \)

Based on our calculation:

\( \rho \) (semi - water) \( \approx 1000 \frac{kg}{m^3} \),

\( v = \frac{\text{flow rate}}{\text{cross section area}} = \frac{900 \mu L/min}{25mm \times 400 \mu m} = 0.0015 \text{ (m/s)} \),

\( L = 400 \mu m = 0.0004 \text{ (m)} \),

\( \mu \) (semi - water) @ 37°C \( \approx 0.0007 \frac{kg}{m.s} \)

So, Reynold’s Number in our device is:

\[ Re = \frac{1000 \times 0.0015 \times 0.0004}{0.0007} = 0.857 < 2300 \rightarrow \text{laminar} \]

Although, our device path’s height is small (400um), path’s width is wide (25mm). So, such designation would help us to use higher flow rate meanwhile benefit from surface tension and still stay in laminar condition.

Cell simulation modelling:

To model cell polarization simulation by CST software, we used Ref. 20 {in manuscript} [1] parameters as reference:

The parameters are:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal cell</th>
<th>Cancer cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma_M$: Medium conductivity (S/m)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>$\sigma_m$: Membrane conductivity (S/m)</td>
<td>5.6E-5</td>
<td>9.1E-6</td>
</tr>
<tr>
<td>$\sigma_cyt$: Cytoplasm conductivity (S/m)</td>
<td>1.31</td>
<td>0.48</td>
</tr>
<tr>
<td>$\varepsilon_M$: Medium Dielectric constant</td>
<td>$80 \varepsilon_0$</td>
<td>$80 \varepsilon_0$</td>
</tr>
<tr>
<td>$\varepsilon_m$: Membrane Dielectric constant</td>
<td>$12.8 \varepsilon_0$</td>
<td>$9.8 \varepsilon_0$</td>
</tr>
<tr>
<td>$\varepsilon_cyt$: Cytoplasm Dielectric constant</td>
<td>$60 \varepsilon_0$</td>
<td>$60 \varepsilon_0$</td>
</tr>
</tbody>
</table>

DEP (in its simplest form) is due to the interaction of a particle’s dipole and the spatial gradient of the electric. One general form of the expression is

$$ F_{DEP} = p \cdot \nabla E $$  \[Eq.1\]

$p$ is the particle’s dipole moment, $\nabla E$ is the gradient of the electric field.

For a uniform sphere placed into a sinusoidal electric field given by $E(r,t) = \text{Re}[E(r) e^{j\omega t}]$, where $E(r)$ is the complex electric-field phasor that contains spatial information on the field intensity and polarization, the overall induced dipole is given by

$$ p(r) = 4\pi \varepsilon_M R^3 \left( \varepsilon_c - \varepsilon_M \right) \cdot K(\omega) \cdot E(r) $$  \[Eq.2\]

where $R$ is the radius of the particle; $\varepsilon_M$ and $\varepsilon_c$ are the complex permittivities of the medium and the cell, respectively, and are each given by $\varepsilon = \varepsilon + \sigma/(j\omega)$, where $\varepsilon$ is the permittivity of the medium or cell, $\sigma$ is the conductivity of the medium or cell, and $j$ is $\sqrt{-1}$. $K$ is known as the Clausius-Mossotti (CM) factor.

In a linearly polarized sinusoidal field, we can combine Equations 1 and 2 to arrive at an expression for the time-average DEP force, given by

$$ \langle F_{DEP}(r) \rangle = \pi R^3 \text{Re}[K(\omega)] \cdot |\nabla E(r)|^2 $$  \[Eq.3\]

where $\langle \rangle$ denotes the time average. If the relative polarizability of the cell is greater than that of the medium, then $\text{Re}[K(\omega)]$ will be positive (known as positive DEP, or pDEP), and the force will be directed up the field gradient. If the cell is less polarizable than the medium, then $\text{Re}[K(\omega)]$ is negative, and the force will be directed down the field gradient (negative DEP, or nDEP). Examining the expression for $K(\omega)$ in Equation 2, one sees that $\text{Re}[K(\omega)]$ can only vary between +1 and −0.5.

Of course, cells are neither uniform (e.g., they are multi-layered particles with a membrane, cytoplasm, etc.) nor necessarily spherical (e.g., red blood cells, some bacteria). These complications do not alter the fundamental physics, but rather result in more complicated expressions for the induced dipole (and the resulting DEP force). The complicated internal cellular structure primarily manifests itself in the Clausius-Mossotti factor ($K$).[2][3]


ANXV/PI Test for viability of the cells exposed to low frequency electric fields

Supplementary Figure 1. Verification of centrifuged cell viability, sample before test. Percentage of viable (Q4), necrotic (Q3), late apoptotic (Q2) and apoptotic (Q1) cells measured by flow citofluorimetry before and after differentiation.

Supplementary Figure 2. Verification of cell viability, sample after test (after stimulation of AC electric field); Percentage of viable (Q4), necrotic (Q3), late apoptotic (Q2) and apoptotic (Q1) cells measured by flow citofluorimetry before and after differentiation.
It is observable that more than 85% of the cells remained alive after stimulation by low frequency AC electric field.

**ANXV/PI Description:**

To detected cell death, Annexin V/PI double staining kit has been used in flow cytofluorimetric analyses. The Annexin V corresponding signal provides a very sensitive method for detecting cellular apoptosis, while propidium iodide (PI) is used to detect necrotic or late apoptotic cells, characterized by the loss of the integrity of the plasma and nuclear membranes. The data generated by flow cytometry are plotted in two-dimensional dot plots in which PI is represented versus Annexin V-FICT. These plots can be divided in four regions corresponding to:

1) **viable cells** which are negative to both probes (PI/FITC -/-; Q4);

2) **apoptotic cells** which are PI negative and Annexin positive (PI/FITC -/+; Q1);

3) **late apoptotic cells** which are PI and Annexin positive (PI/FITC +/+; Q2);

4) **necrotic cells** which are PI positive and Annexin negative (PI/FITC +/-; Q3). [1][2][3]


Comparison between CTC enrichment methods:
*Be noticed that some methods cannot applicable for cancer or CTC separation based on principle.

**Supplementary Table 1. Comparison between cell sorting methods.**

<table>
<thead>
<tr>
<th>Features of the method</th>
<th>Principle</th>
<th>Purity</th>
<th>Recovery</th>
<th>Yield</th>
<th>Efficiency</th>
<th>Cell viability</th>
<th>Sample volume</th>
<th>Throughput (speed)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adherence</td>
<td>Adherence</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>1-5</td>
</tr>
<tr>
<td>Filtration</td>
<td>Size</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>1, 6-9</td>
</tr>
<tr>
<td>Centrifugation</td>
<td>Density</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>1, 10, 11</td>
</tr>
<tr>
<td>Panning</td>
<td>Antibody/ Size</td>
<td>Medium</td>
<td>Medium</td>
<td>Medium</td>
<td>Medium</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>1, 12-16</td>
</tr>
<tr>
<td>MACS</td>
<td>Antibody with Magnetic Beads</td>
<td>High</td>
<td>Medium</td>
<td>Medium</td>
<td>High</td>
<td>Medium</td>
<td>Medium</td>
<td>Medium</td>
<td>1, 17-20</td>
</tr>
<tr>
<td>FACS</td>
<td>Antibody with Fluorescent beads</td>
<td>High</td>
<td>Medium</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>Medium</td>
<td>Medium</td>
<td>1, 21-24</td>
</tr>
<tr>
<td>LMD</td>
<td>Morphological/ Cytochemical/ Antibody</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>No</td>
<td>Small</td>
<td>Low</td>
<td>1</td>
</tr>
<tr>
<td>Optical Tweezer</td>
<td>Hydrodynamics</td>
<td>Medium</td>
<td>High</td>
<td>Medium</td>
<td>Medium</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>25</td>
</tr>
<tr>
<td>Acoustophoresis (Not applicable for Cancer)</td>
<td>Hydrodynamics</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>26</td>
</tr>
<tr>
<td>DEP @ Low Frequency</td>
<td>Polarization/ Dielectric property</td>
<td>Medium</td>
<td>High</td>
<td>Medium</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>This Paper</td>
</tr>
<tr>
<td>DEP @ High Frequency</td>
<td>Polarization/ Dielectric property</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>29, 30</td>
</tr>
</tbody>
</table>

**Highlighted advantages of our system Vs. DEP:**
- DC-based and low frequency DEP with good consideration of safety (joule thermal increment, solvent electrolysis and cell viability) can be more efficient in cell separation.
- DC-based and low frequency DEP are better choice for external samples like cytological samples which they will be useless after analysis.
- There was lack of studies in low frequencies DEP for cancer detection.
- CPF of cells can be a label-free marker in phenotype sorting.
- It is not essential that CTC detection system only detect CTC in blood which is not an early detection. We believe early CTC diagnosis can be detect in liquid biopsy samples which are a transient level between tumor formation and metastasis.
[12] “late as m a n y hybridomas as possible , secreting different monoclonal antibodies directed against a variety of antigens . An answer to this problem is a technique in which hybridomas are selected and cloned in one step imme-