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Modeling and development of a low frequency contactless dielectrophoresis (cDEP) platform to sort cancer cells from dilute whole blood samples

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ABSTRACT

Contactless dielectrophoresis (cDEP) devices are a new adaptation of dielectrophoresis in which fluid electrodes, isolated from the main microfluidic channel by a thin membrane, provide the electric field gradients necessary to manipulate cells. This work presents a continuous sorting device which is the first cDEP design capable of exploiting the Clausius-Mossotti factor at frequencies where it is both positive and negative for mammalian cells. Experimental devices are fabricated using a cost effective technique which can achieve 50 μm feature sizes and does not require the use of a cleanroom or specialized equipment. An analytical model is developed to evaluate cDEP devices as a network of parallel resistor-capacitor pairs. Two theoretical devices are presented and evaluated using finite element methods to demonstrate the effect of geometry on the development of electric field gradients across a wide frequency spectrum. Finally, we present an experimental device capable of continuously sorting human leukemia cells from dilute blood samples. This is the first cDEP device designed to operate below 100 kHz resulting in successful manipulation of human leukemia cells, while in the background red blood cells are unaffected.

1. Introduction

Clinical diagnosis, therapeutics, and comprehensive cell biology benefit from the ability to isolate and enrich rare cells derived from a heterogeneous population (Gossett et al., 2010). Fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) are the most commonly utilized sorting methodologies. These techniques use fluorophore-conjugated antibodies and antibody-conjugated magnetic beads to label and process target cells. Systems employing FACS and MACS provide high throughput screening; however, they have large initial and operational costs, require specialized training, may affect cell fate and function due to shear stress, the use of antibodies, and fluorophores, and requires prior knowledge about cell surface markers (Kumar and Bhardwaj, 2008). Because of this, there is a growing need for a marker-independent isolation and purification method.

A number of marker-independent methods have been developed which sort cells by exploiting unique physical phenomena which can be manipulated on the microscale including streamline manipulation (Takagi et al., 2005), microstructure flow perturbation (Choi et al., 2009), gravity (Warrick et al., 2010), and inertial forces (Di Carlo, 2009). Other methods sort cells based on their intrinsic properties including their volumetric (Vona et al., 2000), mechanical (Mohamed et al., 2009), magnetic (Huang et al., 2008), and electrical (Gascoyne et al., 2009) properties. Recently, Mach et al. demonstrated a massively parallel filtration capable device capable of isolating bacteria from blood with a flow rate of 8 mL/min using inertial forces (Mach and Di Carlo, 2010). Choi et al. were able to isolate cells based on their phase in the cell life cycle in a grooved microfluidic device (Choi et al., 2009). Mohamed et al. demonstrated the ability to isolate circulating cancer cells from whole blood based on size and deformability in a device containing pillars in stages of decreasing pillar-to-pillar spacing (Mohamed et al., 2009). These devices have obvious advantages due to their simplicity and dependence on singular physical phenomenon (i.e., hydrodynamics).

Other methods have recently been reported which improve selectivity to sort cells of similar size, but different genotype by employing electromagnetic forces. Gascoyne et al. demonstrated a tumor cell isolation efficiency of 92% in a dielectrophoresis (DEP) field flow fractionation device using an electric field generated at 60 kHz (Gascoyne et al., 2009). DEP is a phenomenon which occurs at the micro-scale when a dielectric particle is placed in a non-uniform electric field. A net force is generated due to charge distributions within the particle. This has been successfully used

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to examine, manipulate, isolate, or enrich particles (Martinez-Lopez et al., 2009), DNA (Asbury and van den Engh, 1998; Ros et al., 2006), viruses (Muller et al., 1996), and cells (Archer et al., 1999; Hwang et al., 2009; Srivastava et al., 2008). DEP devices typically consist of metal electrodes deposited onto a glass substrate (Yang et al., 1999a). The geometry of these electrodes generates the non-uniform electric field required for DEP. Alternatively, insulator based dielectrophoresis (iDEP) devices employ insulating structures within a microfluidic channel to produce non-uniformities when electrodes are inserted into the ends of the channel (Cummings and Singh, 2003; Lapizco-Encinas et al., 2005). These devices can employ both DC and AC electric fields (Hawkins et al., 2007) and many geometric configurations including sawtooth channels (Chen et al., 2009; Staton et al., 2010).

Contactless dielectrophoresis (cDEP) devices are a new adaptation of this technique in which fluid electrodes, isolated from the main microfluidic channel by a thin membrane, provide the necessary electric field in the sample channel (Shafiee et al., 2009,2010a,b). This helps mitigate some challenges with traditional dielectrophoresis devices including fouling, bubble formation, and electrode delamination (Hughes, 2002). cDEP devices can be fabricated by replication from a single etch master stamp and can be translated to mass fabrication techniques, similar to methods used for iDEP (Sabounchi et al., 2008), while eliminating direct sample-electrode contact. The insulating barriers capacitively couple the fluid electrodes to the sample channel resulting in a complex frequency dependent electric field gradient within the sample channel. The magnitude of the electric field gradient at any frequency is dependent on the geometric and material properties of the device. This work presents a continuous sorting device which is the first cDEP design capable of exploiting the Clausius-Mossotti factor at frequencies where it is either positive or negative for different mammalian cell types. Experimental devices were fabricated using a cost effective fabrication technique which does not require the use of a cleanroom or specialized equipment. An analytical model was developed to evaluate cDEP devices as a network of parallel resistor–capacitor pairs. Two theoretical devices are presented and evaluated using finite element methods to demonstrate the effect of geometry on the development of electric field gradients across a wide frequency spectrum. Finally, we present a third experimental device capable of continuously sorting human leukemia cells from dilute blood samples.

2. Theory

The application of a voltage across conductive and dielectric materials will induce an electric field

$$\vec{E} = -\nabla\phi \tag{1}$$

where ϕ is the applied voltage. Under the influence of this electric field, dielectric particles immersed in a conductive fluid will become polarized. If the electric field is non-uniform, particles are driven towards the regions of field gradient maxima by a translational dielectrophoretic force ($\vec{F}_{\mathrm{DEP}}$) (Pohl and Plymale, 1960)

$$\vec{F}_{\mathrm{DEP}} = \gamma_{\mathrm{DEP}}\nabla\left|\vec{E}\cdot\vec{E}\right| \tag{2}$$

where γ_{DEP} is half the induced dipole moment of the particle. For a spherical particle, this quantity can be represented as:

$$\gamma_{\mathrm{DEP}} = 2\pi \in_{\mathrm{m}} r^3 Re[K(\omega)] \tag{3}$$

where r is the radius of the cell, $\in_{\mathrm{m}}$ is the relative permitivity of the suspending medium, and Re[$K(\omega)$] is the real part of the Clausius-Mossotti (C-M) factor.

$$K(\omega) = \frac{\in_{\mathrm{c}}^* - \in_{\mathrm{m}}^*}{\in_{\mathrm{c}}^* + 2\in_{\mathrm{m}}^*} \tag{4}$$

$$\in^* = \in + \frac{\sigma}{i\omega} \tag{5}$$

where $\in_{\mathrm{c}}^*$ and $\in_{\mathrm{c}}^*$ are the permittivity of the cell and suspending medium respectively, σ is the conductivity, ω is the frequency of the applied field, and $i = \sqrt{-1}$.

A particle independent DEP vector can be defined as

$$\vec{T} = \frac{\vec{F}_{\mathrm{DEP}}}{\gamma_{\mathrm{DEP}}} = \nabla\left|\vec{E}\cdot\vec{E}\right| \tag{6}$$

$$\vec{T} = \nabla\left|(-\nabla\phi)\cdot(-\nabla\phi)\right| \tag{7}$$

$$\vec{T} = \nabla\left|\left(\frac{d\phi}{dx}\right)^2 + \left(\frac{d\phi}{dy}\right)^2 + \left(\frac{d\phi}{dx}\right)^2\right| \tag{8}$$

$$\vec{T} = \begin{bmatrix} \left(\frac{d^3}{dx^3} + \frac{d^3}{dxdy^2} + \frac{d^3}{dxdz^2}\right)\hat{e}_x \\ \left(\frac{d^3}{dx^2dy} + \frac{d^3}{dy^3} + \frac{d^3}{dydz^2}\right)\hat{e}_y \\ \left(\frac{d^3}{dx^2dz} + \frac{d^3}{dy^2dz} + \frac{d^3}{dz^3}\right)\hat{e}_z \end{bmatrix}\phi^2 \tag{9}$$

where $\hat{e}_j$ is a unit vector in the j direction.

Contactless dielectrophoresis devices can be modeled analytically as five resistor-capacitor (R–C) pairs in series. R–C pairs represent the source and sink electrode channels, the two insulating barriers, and the sample channel. The current entering and leaving each of these pairs must be the same and the total impedance of each pair can be calculated using Kirchhoff's current law and Ohm's Law.

$$Z = \frac{X_c^2 R - iX_c R^2}{R^2 + X_c^2} \tag{10}$$

$$X_{\mathrm{c}} = \frac{-1}{\omega C} \tag{11}$$

Z is the total impedance of the resistor–capacitor pair, X_{c} is the capacitive reactance, C is the capacitance, and R is the resistance.

The physical geometry and the material properties of the materials present in this system influence the resistance ($R = \rho L/A$) and capacitance ($C = \in_0 \in_r A/d$) of each element where ρ and $\in_{\mathrm{r}}$ are the resistivity and relative static permittivity of the material respectively, A is the cross-sectional area, L is the length of the resistor and d is the separation distance between two conductive components. It should be noted that for the insulating membranes in a traditional cDEP device, $L = d$.

3. Methods

3.1. Clausius-Mossotti factor analytical model

The Clausius-Mossotti factor for THP-1 human leukemia monocytes and red blood cells (RBC) was modeled over a logarithmic distribution between 100 Hz and 100 MHz using MATLAB (Version R2010a, MathWorks Inc., Natick, MA, USA). Dispersing cytoplasmic properties which effect high frequency behavior were modeled for RBCs as presented by Gimsa et al. (1996). In this method, the conductivity and permittivity of the cell were influenced by an additional dispersion term σ_{c} and $\in_{\mathrm{c}}$ respectively.

$$\sigma_{\mathrm{c}} = \sigma_{\mathrm{co}} + \Delta\sigma\frac{(\omega\tau_{\mathrm{c}})^{2(1-\alpha)}}{(1+\omega\tau_{\mathrm{c}})^{2(1-\alpha)}} \tag{12}$$

$$\in_{\mathrm{c}} = \in_{\mathrm{c}\infty} + \Delta\in_{\mathrm{r}}\left(\frac{1}{(1+\omega\tau_{\mathrm{c}})^{2(1-\alpha)}}\right) \tag{13}$$

$\in_{\mathrm{c}\infty}$ and σ_{co} are the high frequency permittivity and initial conductivity of the cytoplasm, $\Delta\in_{\mathrm{r}}$ and σ_{r} are frequency dependant