Electrodynamic Transport, Electroporation and Lysis of Cells in Pharmacological and Bioanalytical Microsystems

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ABSTRACT

Biological information is stored in cells and is the prime object of interest of pharmacological, biomedical, and bioanalytical professionals. Whatever the interest: elcectroporative drug delivery and pharmacodynamics, in vivo PCR, infection pathology, DNA extraction for amplification and detection the cell membrane barrier needs to be surmounted. In some cases, such as electroporation, in a delicate reversible way, in other e.g. cell lysis in more violent destructive way. This paper presents physiologically consistent computational modeling approach for modeling cell transport and cell interaction with external fields. The overall objective of our effort is to develop comprehensive multiphysics design tools for cell based bioanalytical microsystems. Such microsystems integrate several devices such as cell microfluidic transport and separation, cell lysis, DNA capture and purification, DNA amplification, hybridization, and detection. The aim of this paper is two fold: a) to present new multiscale simulation technique for modeling cell transport in electrically controlled microfluidic devices, and b) to demonstrate it on practical biodevices used for cell trapping and immobilization, cell membrane electroporation, and cell lysis.

Keywords: Electroporation, Cell lysis, Cell membrane, Bio-microreactors, DNA extraction, Bioanalytics.

INTRODUCTION

Recent developments in molecular biology and genetic analysis have inspired strong interests in miniaturization of biotechnology and drug discovery equipment. There is tremendous scientific and commercial competition in developing a complete biochemical intelligent microsystem for extraction, concentration, amplification, analysis, and processing of DNA. We contend that new type microdevices will be demanded for handling cells and macromolecules to support emerging genomic, proteomic, and cellomic disciplines. New technologies will be utilized to integrate microfluidic, biochemical, microelectronic, thermal, and optical devices in a single package, often referred as a "bioChip".

Cell based biosensors hold tremendous promise for drug discovery, gene and protein functionality, cancer therapy, virology, immunology, neurology, and other. Computational modeling of cell physics in fluidic, electrical, chemical environments is a daunting task as cell physiology is determined by an array of dynamic physicochemical processes spatially distributed throughout the cell cytosol, membrane, and the external milieu. In this paper we present a novel 3D Eulerian-Lagrangian computational method for modeling cell transport in microfluidic devices and cell physiological model for modeling cell response to electrical fields.

The aim of this paper is two fold: a) to present new multiscale simulation technique for modeling cell transport in electrically controlled microfluidic devices, and b) to demonstrate it on practical biodevices used for cell trapping and immobilization, cell membrane electroporation, and cell lysis. We model a biological cell and it's components including a lipid bilayer, membrane pores and channels, cytoplasm, and extra cellular matrix. We use fundamental partial differential equations to describe diffusive, convective, and electrokinetic transport of multi-component polyelectrolytes, dynamics of membrane pores, chemical kinetics of metabolic reactions, electric fields, and other phenomena. We present a multi-scale modeling concept in which the cell geometry can be represented by:

- a fine 3D mesh for cell volume and membrane surfaces, to explore sub cellular phenomena, and
- a single polyhedral element with multitude of faces (membrane), to simulate the tissue.

The paper presents details of mathematical formulation, assumptions to model membranes, pores, intracellular transport, electrochemistry and cell interaction with external fields. A novel multi-time-scale modeling method is presented for simulating AC electric field in frequency domain and cell dynamics and physiology in time domain. We demonstrate the CFD-ACE+ tool [1] on microfluidic transport of cells and on two example biochips for cell

membrane electroporation and cell lysis for extraction of DNA

CELL TRANSPORT IN FLUIDIC CHANNELS WITH ELECTROKINETICS

For low concentrations of small particles in fluid flows (particle sizes assumed much smaller than the flow domain) the treatment available in CFD-ACE+ is based on a Lagrangian particle tracking method coupled with an Eulerian solution for the fluid flow. The Eulerian flow field calculations are performed using the pressure-based Navier-Stokes equation solution method [2] used in CFD-ACE+. The flow solutions are then coupled with the particle (cell) mass, momentum and energy equations solved in the Lagrangian frame. Of particular interest for the present project is the momentum equation:

$$\frac{d\mathbf{u}_d}{dt} = \frac{3}{4}\frac{C_D \mu_g R_e}{\rho_d d^2}\left(\mathbf{u}_g - \mathbf{u}_d\right) + \mathbf{g} + F_f \qquad (1)$$

where the left side accounts for the particle acceleration. It includes the drag induced on the particle by the flow and the equal and opposite drag on the flow field. This equation contains the body force $\mathbf{F}_f$ that accounts for all forces induced by physical fields such as electrostatic field. The dielectrophoretic force exerted on the particle by the AC electric field is computed as:

$$\vec{F}_{DEP} = \frac{1}{2}\mathrm{Re}\left[(\vec{m}(\omega)\cdot\nabla)\vec{E}\right] \qquad (2)$$

The Coulomb and dielectric force per unit volume is

$$\vec{F}_E = \rho_q\vec{E} - \frac{1}{2}\vec{E}\cdot\vec{E}\nabla(\varepsilon) + \frac{1}{2}\nabla\left[\rho\frac{\partial\varepsilon}{\partial\rho}\vec{E}\cdot\vec{E}\right] \qquad (3)$$

The particles are tracked in the flow-field using an efficient tracking algorithm The solution procedure involves track calculations for a sufficiently large number of particles/cells through the flow domain, calculation of the source/sink terms in mass, momentum and energy for the flow field and then recalculating the flow-field with the updated source/sink terms. The iterative procedure is continued till convergence, and provides fast solution capability. In the present approach, cell transport equations are solved in a Lagrangian frame of reference moving with the cells, starting from a set of initial conditions. In classical two-phase flow simulations particle size is assumed much smaller than the computational mesh size. Particle is treated as a sniggle point. In microchannels cell size (1-15 microns) is comparable to the channel dimension (tens to hundreds microns) and detailed representation of cell shape has to be modeled. In CFD-ACE+ we use 3D polyhedra elements for cells embedded on the background channel mesh

Figure 1 illustrates the flow pattern in a channel filled with cells predicted with the CFD-ACE+.

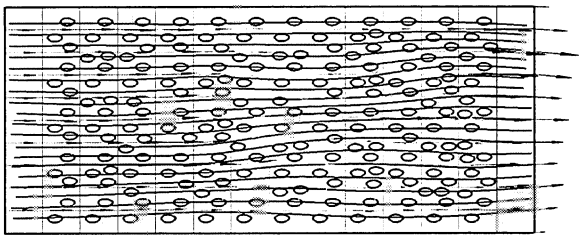

Figure 1. Flow Field of Cells with a Microchannel.

Figure 2 presents simulation results (E field and a snapshot of cell distribution) of dielectrophoretic trapping and electro-rotation of cells in an AC field between four electrodes with phase shift. In the first case (opposite antiphase AC) a low field "hole" is formed in the middle. In the second case (rotating AC) particles rotate following the field.

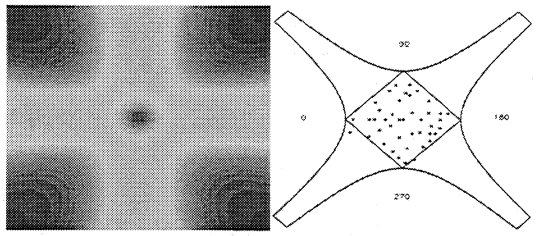

Fig. 2. Electrokinetic Trap and Cell Dynamics in the Trap.

PHYSIOLOGY OF CELL MEMBRANE ELECTROPORATION AND LYSIS

The cell membrane consists of lipid bilayer, which contains large number of receptors, proteins, and fluctuating aqueous pores. The pores are small hydrophobic openings (~1nm size) allowing passage of small neutral particles. When external electric field is applied trans-membrane potential develops and the small hydrophobic pores open up much more by rearranging individual lipids within the pore. This process is known as electroporation. Cell electroporation can be used for a range of therapeutical and bioanalytical procedures including transfection by introduction of DNA, electro-chemotherapy, or even transdermal drug delivery. Figure 3 shows the configuration of continuous membrane and two types of pores.

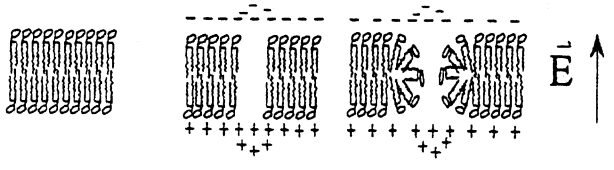

Figure 3. Cell Membrane Continuous Lipid Bilayer and Formation of Hydrophilic and Hydrophobic Pores in the Presence of Applied Electric Field.

The pores stay open long after the removal of E field and provide pathways for the movement of ions, drugs, macromolecules and even DNA fragments into or from the cell. When even larger E field pulses are applied the pores enlarge further up to the (violent) point of cell membrane rapture. In this process, called lysis, interior cell milieu including DNA is released into the surrounding fluids. The released DNA can now be separated, amplified, e.g. by the PCR process [3], and analyzed e.g. by hybridization.
Cell electroporation and lysis, although routinely used in the laboratory, are not well understood, and theoretical models of cell membrane electroporation are beginning to emerge [4-6]. To the authors' knowledge, computational modeling of cell lysis by electric fields has not been reported before.

MODEL OF CELL MEMBRANE ELECTROPORATION AND LYSIS

Biological cells contain cytoplasm with nucleus, cytoskeleton, and large number of macromolecules all suspended in a polyelectrolyte fluid. When exposed to an electric field strong electromigration of charged particles and ions takes place. The transportation of charged ions in the electric field is described by the following equation assuming no chemical reaction exists.

$$\frac{\partial c_i}{\partial t} + \frac{\partial J_{ij}}{\partial x_j} = 0, i = 1,2,3 \cdot N \qquad (4)$$

N is total number of species and the flux J_{ij} is given by Nernst-Planck equation:

$$J_{ij} = u_j c_i - z_i c_i \omega_i E_j - D \frac{\partial c_i}{i \partial x_j} \qquad (5)$$

where c_i i^{th} species concentration, u_j flow velocity, z_i : valance of ions; ω_i: mobility of ion, E_j electric field strength, Di : diffusivity of ions. Fig. 4 shows the electric potential and electric current through the cell for low and high frequency.

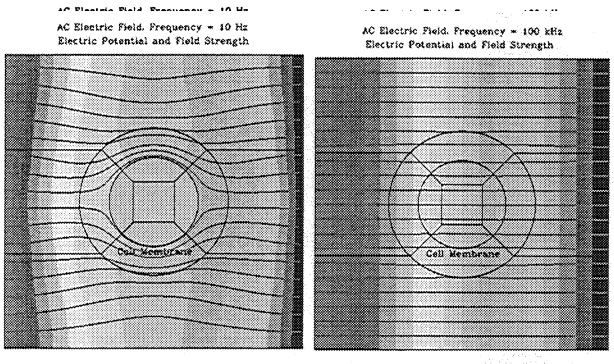

Fig. 4 Biological Cell Exposed to AC field at 10Hz and 10KHz. Electric Field and Current Lines.

At low frequency (10Hz), due to polarization the cell behaves a non-conductor suspended in a conducting medium, and hence most of the current must flow around the cell. In the high frequency (1KHz) inertial of ions is too large and the cell now behaves as a conductor and allows the current to flow through it. Polarization does not take place.

To design a bioanalytical electroporation device one needs to predict minimum energy and impulse required to achieve desired hole size, number density or membrane breakdown for lysis. The energy E(r) required to form a pore, hydrophobic or hydrophilic, is the lesser of the two,

$$E(r) = E_* \left(\frac{r}{r_*} \right) \quad \text{for hydrophobic pore} \qquad (6)$$

$$E(r) = 2\pi\gamma r - \pi\sigma r^2 + \left(\frac{C}{r} \right)^4 \quad \text{for hydrophilic pore} \qquad (7)$$

where, r_* and E_* are the minimum radius and energy barrier for creation of a hydrophilic pore, γ is the pore edge energy, σ is the membrane surface tension, and C is a constant. The energy E(r) corresponds to the situation when there is no applied electric field. In the presence of transmembrane potential V_m, the pore energy denoted by $\varphi(r)$ is given by:

$$\varphi(r) = E(r) - \pi a_p V_m^2 r^2 \qquad \text{where} \qquad (8)$$

$$a_p = \frac{1}{2h} (\varepsilon_w - \varepsilon_m) \varepsilon_0 \qquad (9)$$

where h is the thickness of membrane, ε_w, ε_m are relative permittivities of water and membrane, and ε_0 is the permittivity of a vacuum.

Let n(r,t) denote the pore distribution function: at a given time, t, the number of pores per unit area with a pore radius between r and (r+dr) is n(r,t)dr. The governing equation for n(r,t) is the 5 dimensional (x,y,z,t,r) Smoluchowski PDE equation. We will greatly simplify it by assuming x-y-z-space invariance and integrate over r-space, and define a new term N(t):

$$N(t) = \int_r^\infty n(r,t) dr \qquad (10)$$

The governing ODE equation for N(t) is

$$\frac{dN}{dt} = K \left(1 - \frac{N}{N_{eq}} \right) \qquad (11)$$

where $$K = \alpha \exp\left(\left(V_m / V_{ep} \right)^2 \right) \qquad (12)$$

and $$N_{eq} = N_0 \exp\left(q \left(V_m / V_{ep} \right)^2 \right)$$

The electric potential across the cell membrane and the current density across the membrane are:

$\nabla^2\varphi_i = 0$, $\nabla^2\varphi_e = 0$ intra/extra-cellular space (13)

$$-\vec{n}\cdot(\sigma_i\nabla\varphi_i) = -\vec{n}\cdot(\sigma_e\nabla\varphi_e) = C_m\frac{\partial V}{\partial t} + I_{ion} + I_{ep} \quad (14)$$

where $\vec{n}$ is the normal unit vector of cell membrane, $_i$, and $_e$ are the conductivities of the intracellular and extracellular spaces/media, C_m is the specific membrane capacitance, V_m is the transmembrane potential defined by $V_m = \varphi_i - \varphi_e$, I_{ion} is ionic current, and I_{ep} is the current due to electroporation. These two currents are given by:

$$I_{ion} = g_l(V_m - E_l), \quad I_{ep} = Ni_{ep} \quad (15)$$

Where, g_l is the specific membrane conductance, E_l the reversal potential, i_{ep} is the current through a single pore and N is the pore density defined by equation (10). A detailed formula for i_{ep} can be found in [4]. The cross-sectional area of a single pore is πr_m^2. After Eqn. 11 is solved for the pore density function N, the total cross-sectional area of the pores that allows charged ions to pass through is calculated as $A_p = N\pi r_m^2$, where r_m is the mean pore radius.

The proposed model is used to simulate the cell electroporation and lysis. The cell is modeled as a 3D polyhedral element with several faces and the proposed membrane model is used at each face of the cell. The cell can be stationary or can be in (Lagrangian) motion. The model can be used to predict required optimal AC field intensity, frequency, pulse shape, phase shifting, minimum number of pulses, etc.

The model is demonstrated for electroporation of a simple space-clamped membrane. Fig. 5 shows the time variation of the pore number density, as I_{stim} is first applied for a specified time and removed for both low and high stimulation currents. The results show that for small currents, electroporation is minimal, with very small changes in the pore density from its base value. With larger stimulation current, the electroporation process is much stronger, and increase in pore density is sufficient to lyse the cell.

SUMMARY

The paper presents progress in comprehensive computational modeling of electrodynamic transport, electroporation and lysis of cells in pharmacological and bioanalytical microsystems. The proposed Eulerian-Lagrangian transport model of macro particles (cells) can be used to design biochips. A new 3D model of cell membrane, membrane electroporation and lysis has been presented and demonstrated.

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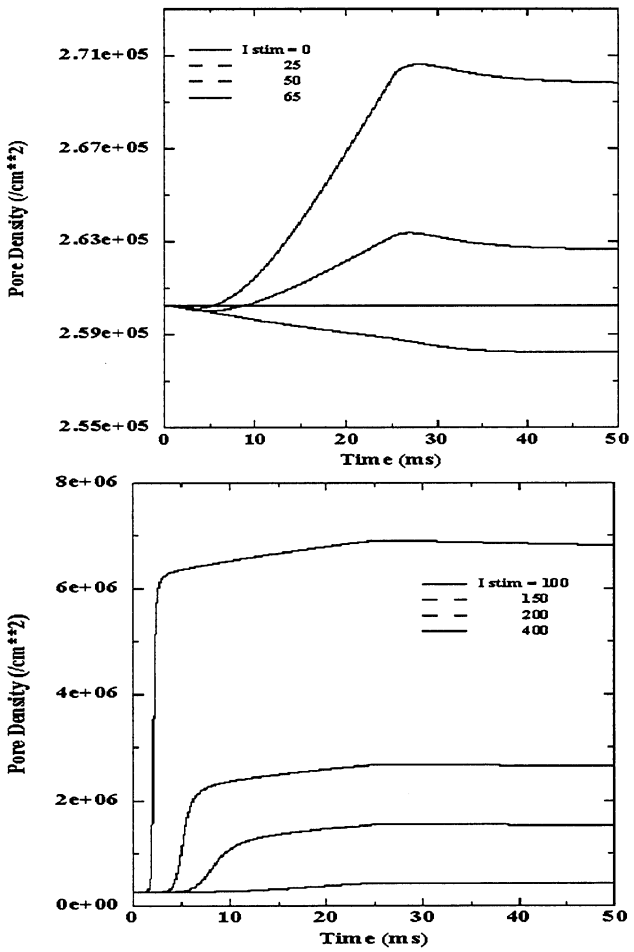

Fig.5 Pore Density in time for low/high Stimulation Current

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