Proceedings of the 28th IEEE
EMBS Annual International Conference
New York City, USA, Aug 30-Sept 3, 2006

Field Stimulation of Cells in Suspension: Use of a Hybrid Finite Element Method

Wenjun Ying*, Navid Pourtaheri and Craig S. Henriquez

Abstract— **Electric fields are used in a range of applications, including gene transfection, electrochemotherapy of tumors and cardiac defibrillation. Despite the widespread use of electric fields, most of the theoretical and computational studies on discrete cellular tissue have focused on a single cell. In this work, we propose a hybrid finite element method to simulate the effects of external electric fields on clusters of excitable cells. The method can be used to model cells of arbitrary cell geometries and non-linear membrane dynamics. The results show that the response of multiple cell, like a single cell, is a two-stage process consisting of the initial polarization that proceeds with cellular time constant (less than one microsecond) and the actual excitation of the cell membrane that proceeds with the membrane time constant (on the order of milliseconds). The results also show that the stimulation of a given cell depends in part on the arrangement of cells within the field and not simply the location within the field, suggesting that classical approaches that ignores the effect of the cells on the field do not adequately predict the cellular response.**

1. INTRODUCTION

The response of a cell to an external electric field is a two-stage process, consisting of the initial polarization that proceeds with cellular time constant (less than one microsecond) and the actual excitation of the cell membrane that proceeds with the membrane time constant (on the order of milliseconds) [1], [2]. It is well-known that the transmembrane potential of a cell stimulated by an external electric field is highly nonuniform, varying from depolarization at the end facing the cathode to hyperpolarization at the end facing the anode. Analytical expressions for the membrane responses have been derived for single cells that are spherical, prolate or oblate spheroidal [3]–[8].

Although the response of a single cell has been studied for various idealized geometries, the response of multiple cells or cells in suspension to an electric field is less well understood. The induced transmembrane potential inside a multiple cell system depends on not only cell density but also on the arrangement and position of cells [9], [10] in the field. In the literature, the corresponding field stimulation theory for multiple and possibly excitable cells is limited. The electric potential distribution within a cell suspension can only be studied by numerical methods, including the finite difference, finite element methods and their variants such as the equivalent circuit method [11]–[13], the transport lattice method [14], [15], the impedance method [16], [17] and the boundary element method [18]–[20].

In this work, we present a hybrid finite element method to compute the response of multiple excitable cells in an external electric field. The advantage of the approach is the ability to model cells in suspension with varying cell sizes, geometries, membrane dynamics and positions in space. The simulation results show that positions and magnitudes of the maximum and minimum potentials of the membrane potential depend on the arrangement of the cells, and thus the cellular response cannot be predicted by a model that ignores the perturbation of the cells on the field itself.

2. METHODS

A. Model

We consider a discrete cellular tissue in an external electric field $\mathbf{E}$. The electrodes to generate the electric field are located far away from the cellular tissue. We assume that the cell membrane Γ has negligible thickness since, under normal conditions, the cell membrane thickness is several orders smaller than the dimensions of the extracellular space Ω_e and the intracellular space Ω_i, occupied by cytoplasm. We also assume the intra- and extracellular media have homogeneous conductivities: both the intracellular conductivity σ_i (mS/cm) and the extracellular conductivity σ_e (mS/cm) are constant. Under these assumptions, the intra- and extracellular potentials Φ_i (mV) and Φ_e (mV) satisfy the Laplace's equation:

$$\triangle\Phi_i = 0 \qquad \text{in } \Omega_i, \tag{2.1a}$$

$$\triangle\Phi_e = 0 \qquad \text{in } \Omega_e. \tag{2.1b}$$

On the cell membrane, the electric potential is discontinuous and the difference between intra- and extracellular potentials is the transmembrane potential V_m:

$$\Phi_i - \Phi_e = V_m. \tag{2.2}$$

The electric flux through the membrane is continuous:

$$-\sigma_i \frac{\partial \Phi_i}{\partial \mathbf{n}} = -\sigma_e \frac{\partial \Phi_e}{\partial \mathbf{n}} \equiv \lambda. \tag{2.3}$$

Here, $\mathbf{n}$ denotes the unit normal on the membrane pointing to the extracellular space. We denote the electric flux through the membrane by λ. In addition, due to the assumption on the location of electrodes, the negative gradient of the extracellular potential far away from the cellular tissue corresponds to the uniform electric field:

$$-\nabla\Phi_e \to \mathbf{E} \qquad \text{as } |\mathbf{x}| \to \infty. \tag{2.4}$$

This work was supported in part by NIH grant R01HL76767.

Wenjun Ying and Navid Pourtaheri are with the Department of Biomedical Engineering, Duke University, Durham NC 27708, USA *ying@cel-mail.bme.duke.edu*

Craig S. Henriquez is with the Departments of Biomedical Engineering and Computer Science, Duke University, Durham, NC 27708, USA *ch@cel-mail.bme.duke.edu*

On the other hand, by the biophysical property of cell membrane, the electric flux λ equals the membrane current I_m ($\mu A/cm^2$), which is the sum of ionic and capacitive currents:

$$\lambda(V_m) = C_m \frac{\partial V_m}{\partial t} + I_{ion}(\mathbf{V}_m). \tag{2.5}$$

Here, C_m is the membrane capacitance per unit area ($\mu F/cm^2$) and I_{ion} is the ionic current that consists of several components carried by different ions. In this work, the Hodgkin-Huxley model is used to describe the ionic current [21].

B. Asymptotic Analysis

Assume that the diameter of each cell in the discrete cellular tissue is small compared with the extracellular region. The dimensional analysis and perturbation method [2] is still applicable. The response of the cells is a two-stage process, consisting of the initial polarization that proceeds with cellular time constant (less than one microsecond) and the actual excitation of the cell membrane that proceeds with the membrane time constant (on the order of milliseconds).

In the initial polarization stage, a cell behaves as a combination of a monopole and a dipole. The electric flux through the cell membrane dominates the ionic current since the cell membrane is asymptotically non-conductive in the phase. So, the membrane current consists of the capacitive current only. The relationship (2.5) of membrane current and electric flux reduces to

$$C_m \frac{\partial V_m}{\partial t} = -\sigma_i \frac{\partial \Phi_i}{\partial \mathbf{n}}. \tag{2.6}$$

In the excitation stage, a cell behaves mainly as a dipole. The electric flux on both sides of the membrane Γ are essentially diminishing, i.e.,

$$\sigma_i \frac{\partial \Phi_i}{\partial \mathbf{n}} = \sigma_e \frac{\partial \Phi_e}{\partial \mathbf{n}} = 0 \qquad \text{on } \Gamma. \tag{2.7}$$

Thus, as indicated by (2.5), the capacitive current balances the ionic current on each membrane patch. The time course of the transmembrane potential simply follows the ODE:

$$C_m \frac{\partial V_m}{\partial t} = -I_{ion}(V_m). \tag{2.8}$$

Note that, both the intra- and extracellular potentials are uniquely determined by the Laplace's equation (2.1), the far field boundary condition (2.4) and homogeneous Neumann boundary conditions (2.7), but up to an additive constant. This implies that the extracellular potential Φ_e is time-independent in the excitation phase, and the intracellular potential Φ_i inside each cell is spatially uniform. The value of intracellular potential Φ_i can be calculated by simply adding transmembrane potential V_m and extracellular potential Φ_e.

On the other hand, according to the current-flux relationship (2.6) in the initial polarization phase, the Gauss theorem indicates that

$$\int_{\Gamma_k} C_m \frac{V_m}{\partial t}\, ds = -\int_{\Gamma_k} \sigma_i \frac{\partial \Phi_i}{\partial \mathbf{n}}\, ds = 0, \tag{2.9}$$

where Γ_k is the membrane of the k^{th} cell in the cellular tissue. This means the average transmembrane potential on each cell membrane is constant in the period. Let V_{rest} be the resting membrane potential before the electric field is turned on. As a result of (2.9), at the end of the initial polarization phase, the spatially uniform intracellular potential Φ_i can be evaluated by

$$\Phi_i = V_{rest} + \frac{1}{|\Gamma_k|}\int_{\Gamma_k} \Phi_e\, ds,$$

and the transmembrane potential on the membrane Γ_k:

$$V_m = V_{rest} + \frac{1}{|\Gamma_k|}\int \Phi_e\, ds - \Phi_e. \tag{2.10}$$

In fact, the expression (2.10) also indicates that one end of the cell must be depolarized and another end must be hyperpolarized. Furthermore, the maximum transmembrane potential on the cell membrane Γ_k can be approximated by

$$\begin{aligned} V_m^{(max)} &= V_{rest} + \frac{1}{|\Gamma_k|}\int_{\Gamma_k} \Phi_e\, ds - \Phi_e^{(min)} \\ &\approx V_{rest} + \frac{1}{2}(\Phi_e^{(max)} - \Phi_e^{(min)}). \end{aligned} \tag{2.11}$$

Here, $|\Gamma_k|$ denotes the size of the membrane Γ_k, and $\Phi_e^{(max)}$ and $\Phi_e^{(min)}$ are the local extrema of Φ_e on Γ_k. It is known that the activation or inactivation of a cell depends on the maximum transmembrane potential $V_m^{(max)}$ at the end of the initial polarization phase. A cell will be activated in the excitation phase if and only if the maximum transmembrane potential $V_m^{(max)}$ is greater than some threshold value. By the linear dependence (2.11) of $V_m^{(max)}$ on the local extrema of Φ_e, we may conclude that the extracellular potential Φ_e that solves the Laplace's equation with the no-flux membrane condition (2.7) determines the activation or inactivation of cells in a discrete cellular tissue.

C. Numerical Methods

It can be demonstrated by Green's identities that, given the transmembrane potential V_m, the intra- and extracellular potentials Φ_i and Φ_e are, up to an additive constant, uniquely determined by the Laplace's equation (2.1) and the boundary conditions (2.2)-(2.4). Consequently, the electric flux λ through the membrane is uniquely determined by the transmembrane potential V_m. In fact, the electric flux λ is a linear function of the transmembrane potential V_m. In this sense, the equation (2.5) can be regarded as an ordinary differential equation (ODE) with respect to the transmembrane potential V_m. Given appropriate initial values for the transmembrane potential V_m, its time course is thus uniquely determined by the ODE (2.5).

However, due to the complexity of cell geometry and the possibly complicated arrangement of cells, it is generally difficult to give an analytical expression for the electric flux λ in terms of the transmembrane potential V_m. In our approach, we approximate the electric flux λ by solving the boundary value problem (2.1)-(2.4) with a hybrid finite element method [22]. Unlike the standard finite element

method, the hybrid finite element method introduces the electric flux on the membrane as an independent variable into the finite element system, which yields more efficient and accurate approximation.

Moreover, once the electric flux λ is computed, we may regard it as a parameter in the ODE (2.5). Then the ODE (2.5) can be integrated by a standard ODE solver such as the Euler and Runge-Kutta methods.

Let Δt be the time step for integrating the ODE (2.5) and $t^n = n\Delta t$ with integer $n \geq 0$. Suppose that the cells are initially at rest, i.e., the transmembrane potential is equal to the resting potential V_{rest} at $t = 0$. The algorithm used for modeling the response of cellular tissue to the external electric field $\mathbf{E}$ consists of two steps:

- **Step 1.** *With the old transmembrane potential V_m at $t = t^n$, we obtain the electric flux λ by numerically solving the membrane problem (2.1)-(2.4) with the hybrid finite element method.*
- **Step 2.** *With the old transmembrane potential V_m at $t = t^n$ and the membrane current λ obtained in* **Step 1**, *we integrate the ODE (2.5) by a time step Δt with a standard ODE solver to get new values of V_m at $t = t^{n+1}$.*

Repeat these two steps above until the final computational time is reached.

3. RESULTS

Classical approaches to field stimulation ignore the perturbation of the field by the cells and assume that the cell's location in the field determines the membrane response. Two simulations were performed to investigate the effect of the cell position on excitation. In both simulations the electric field was assumed to be $\mathbf{E} = 4\,V/cm$, the conductivity of the intracellular and extracellular spaces were $\sigma_i = 5\,mS/cm$ and $\sigma_e = 20\,mS/cm$, respectively and membrane capacitance was $C_m = 1\,\mu F/cm^2$. The electric field is generated by two planar electrodes with the anode on the left and the cathode on the right.

In the first experiment, the cellular tissue consists of eight circular cells only. Each cell has the same diameter, $12.5\,\mu m$. As shown in Fig. 1, the cells in the center column do not all have the same membrane response despite being located in the middle of the field. Cell F is brought above threshold while Cell A is not (see Fig. 1 (c) and (d)). It is observed that only cells F and H are fully activated during the stimulation. In addition, the results also show that the locations of the maximum and minimum membrane potentials (marked by squares in Fig. 1 (b)) are not mirrored relative to the central axis of the cell. The response of the transmembrane potential depends on the cell arrangement as well as the cell location.

In the second experiment, a field was applied to 139 randomly distributed cells in suspension. Note that each cell in the tissue has the same size with diameter equal to those in the first simulation. The electric potential distribution around the cells, as shown in Fig. 2 (b), is highly non uniform and locations of the maximum and minimum potentials is again highly dependent of the position of the cell and its proximity to other cells.

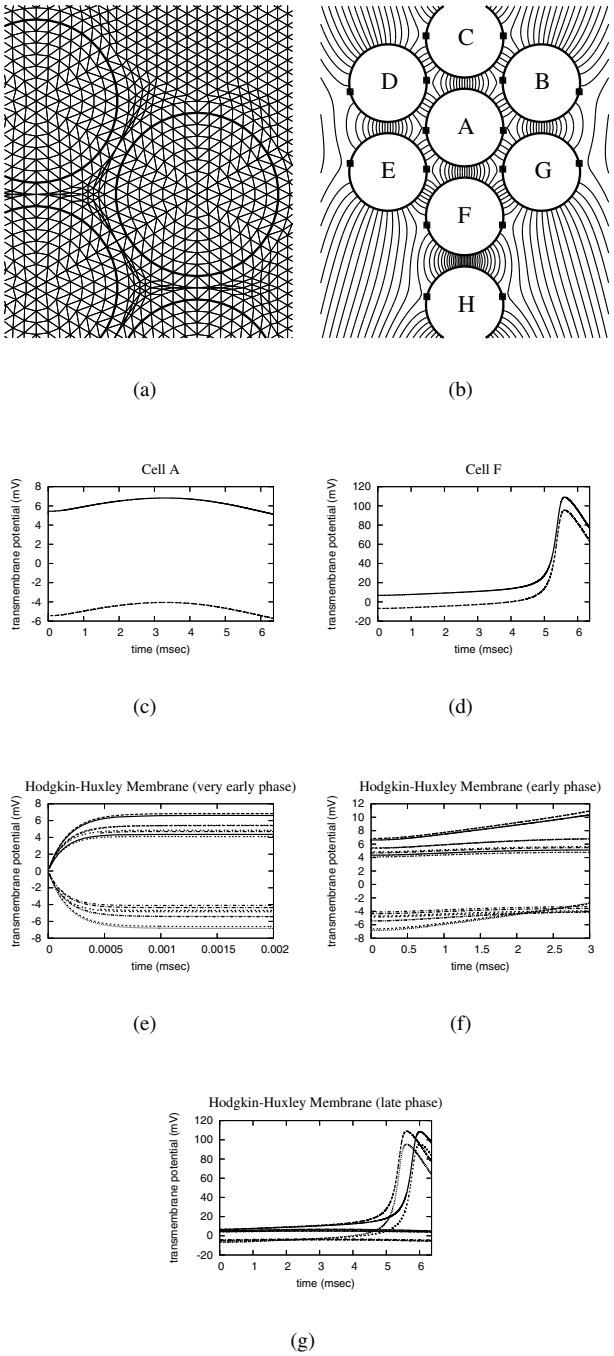
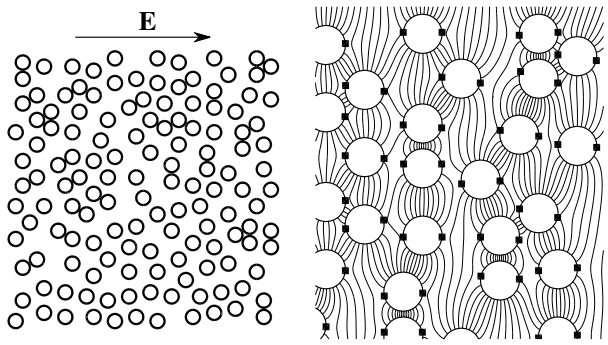


Fig. 1. Field stimulation of the eight cells in suspension: (a). local triangular grid used by the hybrid finite element method; (b). isopotential lines at a time in the excitation phase (no isolines are plotted for the spatially uniform intracellular potential); (c). time course of transmembrane potential at extreme points of cell A (marked by squares in (b)); (d). time course of transmembrane potential at extreme points of cell F (marked by squares in (b)); (e). time course of transmembrane potential at extreme points of all cells (marked by squares in (b)) in the initial polarization; (f). time course of transmembrane potential at extreme points of all cells (marked by squares in (b)) in the early excitation phase; (g). time course of transmembrane potential at extreme points of all cells (marked by squares in (b)) in the late excitation phase.



(a) (b)

Fig. 2. Field stimulation of the 139-cell in suspension: (a). 139 randomly generated discrete circular cells of equal size; (b). isopotential lines of the extracellular potential around the cells on the upper-right corner of the tissue (no isoline could be plotted for the intracellular potential since it is spatially uniform within each cell).

4. CONCLUSION

In this paper, we applied a hybrid finite element method for modeling the possibly non-linear response of a cell or cluster of cells in suspension to an external electric field. The method is able to account for the two-stage response, consisting of the initial polarization that proceeds with cellular time constant (less than one microsecond; see Fig. 1 (e)) and the actual excitation of the cell membrane that proceeds with the membrane time constant (on the order of milliseconds). The simulations also showed that the presence of the cells has a significant impact on the local potential and current distributions. As a result, the location of a cell in the field does not determine the nature of the response nor the locations of the maximum and minimum potential values on the cell membrane. Because a variety of the nonlinear effects can be incorporated, this application of the hybrid finite element method to biological cells can be used to help design strategies for more effective drug and gene delivery [23], or neural/cardiac stimulation using electric fields.

REFERENCES

[1] L. Tung and J. R. Borderies, "Analysis of electric field stimulation of single cardiac muscle cells," *Biophysical Journal*, vol. 63, no. 2, pp. 371–386, 1992.

[2] W. Krassowska and J. C. Neu, "Response of a single cell to an external electric field," *Biophysical Journal*, vol. 66, pp. 1768–1776, 1994.

[3] H. Fricke, "The electric permittivity of a dilute suspension of membrane-covered ellipsoids," *J. Appl. Phys.*, vol. 24, pp. 644–646, 1953.

[4] H. P. Schwan, "Electrical properties of tissue and cell suspensions," *Adv. Biol. Med. Phys.*, vol. 5, pp. 147–209, 1957.

[5] T. Kotnik and D. Miklavcic, "Analytical description of transmembrane voltage induced by electric fields on spheroidal cells," *Biophysical Journal*, vol. 79, no. 2, pp. 670–679, 2000.

[6] J. Gimsa and D. Wachner, "Analytical description of the transmembrane voltage induced on arbitrarily oriented ellipsoidal and cylindrical cells," *Biophysical Journal*, vol. 81, no. 4, pp. 1888–1896, 2001.

[7] B. Valic, M. Golzio, M. Pavlin, A. Schatz, C. Faurie, B. Gabriel, J. Teissie, M. P. Rols, and D. Miklavcic, "Effect of electric field induced transmembrane potential on spheroidal cells: theory and experiment," *Eur. Biophys. J.*, vol. 32, no. 6, pp. 519–528, 2003.

[8] D. C. Lee and W. M. Grill, "Polarization of a spherical cell in a nonuniform extracellular electric field," *Annals of Biomedical Engineering*, vol. 33, no. 5, pp. 603–615, 2005.

[9] R. Susil, D. Semrov, and D. Miklavcic, "Electric field induced transmembrane potential depends on cell density and organization," *Electro. Magnetobiol.*, vol. 17, no. 3, pp. 391–399, 1998.

[10] M. Pavlin, N. Pavselj, and D. Miklavcic, "Dependence of induced transmembrane potential on cell density, arrangement and cell position inside a cell system," *IEEE Trans. Biomed. Eng.*, vol. 49, no. 6, pp. 605–612, 2002.

[11] E. C. Fear and M. A. Stuchly, "Modeling assemblies of biological cells exposed to electric fields," *IEEE Trans. Biomed. Eng.*, vol. 45, no. 10, pp. 1259–1271, 1998.

[12] ——, "A novel equivalent circuit model for gap-connected cells," *Phys. Med. Biol.*, vol. 43, no. 6, pp. 1439–1448, 1998.

[13] A. Ramos, A. Raizer, and J. L. Marques, "A new computational approach for electrical analysis of biological tissues," *Bioelectrochemistry*, vol. 59, no. 1-2, pp. 73–84, 2003.

[14] T. R. Gowrishankar and J. C. Weaver, "An approach to electrical modeling of single and multiple cells," *Proc. Natl. Acad. Sci. USA*, vol. 100, no. 6, pp. 3203–3208, 2003.

[15] D. A. Stewart, T. R. Gowrishankar, K. C. Smith, and J. C. Weaver, "Cylindrical cell membranes in uniform applied electric fields: validation of a transport lattice method," *IEEE Trans. Biomed. Eng.*, vol. 52, no. 10, pp. 1643–1653, 2005.

[16] M. A. Stuchly and M. Xi, "Modeling induced currents in biological cells exposed to low-frequency magnetic fields," *Phys. Med. Biol.*, vol. 39, no. 9, pp. 1319–1330, 1994.

[17] J. F. DeFord and O. P. Gandhi, "An impedance method to calculate currents induced in biological bodies exposed to quasi-static electromagnetic fields," *IEEE Trans. Electromagn. Compat.*, vol. BME-27, pp. 168–173, 1985.

[18] L. J. Leon and F. A. Roberge, "A new cable model formulation based on Green's theorem," *Ann. Biomed. Eng.*, vol. 18, no. 1, pp. 1–17, 1990.

[19] ——, "A model study of extracellular stimulation of cardiac cells," *IEEE Trans. Biomed. Eng.*, vol. 40, no. 12, pp. 1307–1319, 1993.

[20] K. R. Foster and A. E. Sowers, "Dielectrophoretic forces and potentials induced on pairs of cells in an electric field," *Biophysical Journal*, vol. 69, no. 3, pp. 777–784, 1995.

[21] A. L. Hodgkin and A. F. Huxley, "A quantitative description of membrane current and its application to conduction and excitation in nerve," *Journal of Physiology*, vol. 117, pp. 500–544, 1952.

[22] W. J. Ying and C. S. Henriquez, "Hybrid finite element method for field stimulation of biological cells," 2006, submitted.

[23] E. Neumann, S. Kakorin, and K. Toensing, "Fundamentals of electroporative delivery of drugs and genes," *Bioelectrochem. Bioenerg.*, vol. 48, no. 1, pp. 3–16, 1999.