Journal of Theoretical Biology 360 (2014) 83–94

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

Conducting and permeable states of cell membrane submitted to high voltage pulses: Mathematical and numerical studies validated by the experiments

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HIGHLIGHTS

- We differentiate conductive and permeable states of a cell membrane.
- We follow concentrations of markers uptaken by permeabilized cells.
- Numerical methods and a 3D code have been specifically written to provide results.
- *in vitro* experimental results validate qualitatively our model.

ARTICLE INFO

Article history:
Received 4 February 2014
Received in revised form
11 June 2014
Accepted 22 June 2014
Available online 8 July 2014

Keywords:
Cell modeling
Non-linear partial differential equations
Finite differences on cartesian grid
Surface diffusion discretization

ABSTRACT

The aim of this paper is to present a new model of *in vitro* cell electropermeabilization, which describes separately the conducting state and the permeable state of the membrane submitted to high voltage pulses. We first derive the model based on the experimental observations and we present the numerical methods to solve the non-linear partial differential equations. We then present numerical simulations that corroborate qualitatively the experimental data dealing with the uptake of propidium iodide (PI) after millipulses. This tends to justify the validity of our modeling. Forthcoming work will be to calibrate the parameters of the model for quantitative description of the uptake.

1. Introduction

Electroporation is a destructuration of a cell membrane organization leading to an increase of permeability to molecules that usually do not diffuse across the membrane. Even though the increase of membrane permeability is a consequence of the electric shock, the internalization of molecules into the cytoplasm cannot be described by the conducting state of the membrane. More precisely, it has been experimentally observed that the cell membrane may remain permeable several minutes after the electric pulses delivery, while experiments by Benz et al. (1979) have reported that the membrane conductivity almost recovers its steady value within several microseconds after the end of the pulse. Therefore it is important, from the modeling point of view, to distinguish the electric phenomenon, which leads to the increase of membrane conductivity, from the transport of molecules across the permeable membrane. This transport can be obtained by different ways, depending on the molecules: small molecules, which do not interact neither with the membrane nor with the cytoskeleton, can diffuse into the cytoplasm, while active transport (such as ramping process on the membrane or transport due to electrophoretic forces) are needed to make large molecules such as DNA enter the cell.

An electrodiffusion model was already proposed and studied by Smith and Weaver (2012), but it is restricted to the 1D case and the coupling between electroporation and transport across the membrane was not considered. Here we provide a model that describes the *in vitro* process of the internalization of extracellular molecules into the cell, thanks to the application of high amplitude pulses. Our model is based on a non-linear system of partial differential equations, and the numerical results are obtained for 3-dimensional cells.

Even though it is well-known by experimenters that the high conducting state and the high permeable state of the membrane

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http://dx.doi.org/10.1016/j.jtbi.2014.06.027
0022-5193/

do not coincide, the current models of electroporation do not distinguish these two states. For instance, the currently most achieved model of DeBruin and Krassowska (1999), Neu and Krassowska (1999, 2006), and Smith et al. (2004) only describes the electrical potential in the cell. Their modeling leads to membrane conducting state, which lasts several seconds (see DeBruin and Krassowska, 1999, Fig. 7). Such a duration is smaller than the permeable state duration observed by experiments with bleomycin – which still enters the cell several minutes after the end of the pulse – but much longer than the duration of the conducting state of the membrane, which stays highly conducting during several microseconds according to Benz et al. (1979). For all these reasons, the current models were not satisfactory. In this paper, we propose a new model, which differentiates the conducting state from the permeable state of the membrane, and we show that the simulations corroborate the experimental data.

The paper is organized as follows. In the next section, we present generically the system of partial differential equations, which will be used to model the cell electropermeabilization. We then clarify the assumptions on which is based the model, and we derive the non-linear law that accounts for the change in the conducting and permeable states of the membrane. We then present numerical methods that make it possible to simulate accurately the electric field and the transport of the molecules from the extracellular domain into the cell cytoplasm. We end by numerical simulations that corroborate qualitatively the different experimental observations.

2. Statement of the generic partial differential equations

In this section, we briefly present the main partial differential equations that describe the phenomenon. Roughly speaking, it consists of a Poisson equation for the electric potential and a diffusion-transport equation for the non-permeant molecules. In Section 3, we will focus on the non-linearity due to the electropermeabilization.

2.1. Geometry, notations

The cytoplasm $\mathcal{O}_c$ and the extracellular medium $\mathcal{O}_e$ are considered as homogeneous materials with respective conductivities (see Fig. 1):

$$\sigma = \begin{cases} \sigma_e & \text{in } \mathcal{O}_e, \\ \sigma_c & \text{in } \mathcal{O}_c. \end{cases}$$

We denote by Γ the boundary of $\mathcal{O}_c$ which is supposed to be smooth. Let $\Omega = \mathcal{O}_e \cup \mathcal{O}_c \cup \Gamma$ be the whole domain, and $\partial\Omega$ its boundary. It is worth noting that Γ is assumed to be fixed and thus we do not consider a free-boundary problem. Variations of the volume due to change of osmolarity are not in the scope of the present paper. We refer to Poignard et al. (2011), for more details.

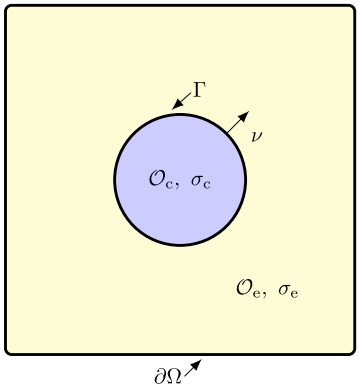

Fig. 1. Scheme of the cell embedded in the extracellular domain.

The membrane is thus described by the single interface Γ with no thickness, and ν designates the unit normal vector to Γ, outward from $\mathcal{O}_c$. The flux of a function f across Γ is noted as $\partial_\nu f|_{\Gamma^+}$ or $\partial_\nu f|_{\Gamma^-}$ depending on the side of the interface, respectively $\mathcal{O}_e$ for Γ^+ and $\mathcal{O}_c$ for Γ^-. We use the following notation for the jump of a function f across the interface:

$$[f]_\Gamma = f|_{\Gamma^+} - f|_{\Gamma^-}.$$

2.2. Electric potential

The electric potential is governed by the following equations:

$$\Delta u = 0 \quad \text{in } \mathcal{O}_c \cup \mathcal{O}_e, \tag{1a}$$

$$\sigma_e \partial_\nu u|_{\Gamma^+} = \sigma_c \partial_\nu u|_{\Gamma^-}, \tag{1b}$$

$$C_m \partial_t [u]_\Gamma + S_0([u]_\Gamma - u_0) + S_{ep}(t,[u]_\Gamma)[u]_\Gamma = \sigma_c \partial_\nu u|_{\Gamma^-}, \tag{1c}$$

$$u(t,\cdot)|_{\partial\Omega} = u_{imp}(t,\cdot), \quad u(0,\cdot) = u_0, \tag{1d}$$

where S_0 is the resting membrane conductivity, u_0 is the resting potential and u_{imp} is the boundary condition determined by the pulse. Eq. (1b) corresponds to the continuity of the electric current through the membrane. Eq. (1c) is a Kirchhoff law, where the $C_m \partial_t [u]_\Gamma$ term represents the capacitive effect of the membrane and $S_{ep}(t,[u]_\Gamma)[u]_\Gamma$ is the electroporation current.

The description of the conducting state of the membrane is obtained by imposing a nonlinear law on S_{ep} that will be described in the next section. Note that the term $S_{ep}(t,[u]_\Gamma)[u]_\Gamma$ corresponds to the electroporation current of DeBruin and Krassowska (1999):

$$I_{ep} = N_{ep}(t,[u]_\Gamma) i_{ep}([u]_\Gamma),$$

after linearization of the current through one pore $i_{ep}([u]_\Gamma)$. However, we emphasize that the characteristic time of pore creation of Neu and Krassowska's model depends on the membrane voltage instead of being intrinsic to the membrane. Moreover pore density N_{ep} is not bounded (DeBruin and Krassowska, 1999) which is hardly defensible from the physical point of view, and therefore we prefer to change it into a sliding-door model given in Section 3.

2.3. Diffusion and electric transport of non-permeant molecules

Since the experimental data on electropermeabilization is mainly based on the internalization of non-permeant molecules into cells or vesicles, such as propidium iodide (PI)[1] or DNA, we also describe the motion of these molecules around and inside the cell. This model must take into account the two main modes of propagation of these molecules: the diffusion for small molecules such as PI and the electrophoresis for charged molecules such as DNA. We assume that the electrophoretic forces given by $-\mu_e \nabla u$ holds only in the outer medium, with μ_e being the electrical motility of the molecule M in $\mathcal{O}_e$. This assumption is plausible since the electric field in the cytoplasm is very low due to the shielding effect of the membrane, and since the cytoplasm is composed of cytoskeleton and organelles, which prevents the diffusion and the electric transport of large molecules inside the cell.

We suppose that at the initial time, the concentration of M is constant and equal to M_0 in $\mathcal{O}_e$ while it is set to zero in $\mathcal{O}_c$.

[1] PI is a small molecule which is fluorescent inside the cytoplasm of the cell. It is thus a good fluorescent marker of membrane electropermeabilization.