Cimpa School
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On mathematical models for Permeabilization of cells in an Electric field
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"Classical" Electropermeabilization Modeling at the Cell Scale

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ANR project MEMOVE, including other collaborators

- Frédéric de Gournay (Toulouse)
- Lluis Mir & Aude Silve (CNRS, Institut Gustave Roussy, Villejuif)
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Today’s talk

On Cells and Membranes
What is electropermeabilization?
Mathematical questions
A molecular dynamics approach
A model for the membrane
Schwan’s model
A static model
A dynamic model
On Cells and Membranes
A cell consists of a membrane surrounding the cytoplasm. A typical, and maybe the simplest, example is a vesicle: here the green head + the yellow tail represent a phospholipid.

[Diagram of a vesicle with labeled parts: Hydrophilic head, Aqueous solution, Hydrophobic tail]
On Cells and Membranes

- The membrane is a barrier protecting the cytoplasm against intrusions, and filters the ion exchange between the inside and the outside.

- It is composed of a thin **phospholipidic bilayer**, with a few transmembrane proteins.

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**Diagram:**

- **Phospholipids**
- **Transmembrane proteins**

- The membrane thickness is **5nm**.
On Cells and Membranes

- A phospholipid has a « fatty » tail, which is hydrophobic, and a hydrophilic head which is an electric dipole.

- This dipole may rotate around the axis of the tail, to some extent.

- Relative sizes are as follows:
  - Cell’s diameter ~ 5 000 nm
  - Membrane thickness ~ 5 nm
  - Distance between two cells ~ 100 nm
  - Distance between two phospholipids ~ 1 nm
  - A molecule of water ~ 0.1 nm
On Cells and Membranes

A typical example of a phospholipid is
(image from http://www.uic.edu/classes/bios/bios100/lecturesf04am/lect08.htm)
On Cells and Membranes

The schematic scales involved can be depicted as follows:
(图来自http://www.britannica.com/EBchecked/topic/457489/phospholipid)
What is electropermeabilization?
What is electropermeabilization?

- It has been observed (R. Stampfli 1958, E. Neumann & K. Rosenheck 1972) that the cell’s membrane reacts in a special manner to an intense electric field and its permeability changes.

- Before any electric field: no molecule may traverse across the bilayer.

- In particular for a cell membrane this means that no external molecules may enter the cell.
What is electropermeabilization?

- After an electric field (here parallel to the tails) is imposed: some molecules may traverse the bilayer, as if a « pore » had appeared.

For a cell membrane this means that some external molecules do enter the cell.

Such a phenomenon may be of interest in order to deliver molecules of drugs or genes into the cell.
What is electropermeabilization?

- As a matter of fact, one may imagine two kinds of « pores »

![Diagram of hydrophobic and hydrophilic pores](a) Hydrophobic pore (b) Hydrophilic pore

- However it is not known whether there is creation of « pores », or rather there is a « permeabilization » process under the intense electrical field...
What is electropermeabilization?

- This is a very important phenomenon, since through the pore molecules may enter the cell.

- Based on this, a new treatment of cancer tumors has been set up by several teams around the world (among others Lluis Mir in France, Julie Gehl in Denmark):

  electrochemotherapy

- A drug, such as bleomycin, is injected to the patient, locally or intravenously. Within 8 to 12 minutes one creates around the tumor a strong electric field, with several short pulses (from microsecond to nanosecond durations).

- It is expected that the membranes of the cells in the tumor will be more permeable, and thus the drug and some molecules of bleomycin would enter into such cells.
What is electropermeabilization?

The process of *electropermeabilization*, or *electroporation*, is assumed to be as follows:

(a) Initial configuration
(b) High short pulses
(c) Interruption of the pulses
What is electropermeabilization?

Principle of electrochemotherapy (according to Damian Miklavčič)

Electrochemotherapy

- Electric pulse generator
- Electrodes
- Tumour
- Systemic or intratumoural drug injection
- Electric pulse application
- Anticancer drug surrounds the cells
- Increased membrane permeability allows access to the cytosol
- Membrane reseals, anticancer drug exerts its cytotoxicity
What is electropermeabilization?

The typical apparatus used is as follows (according to Lluis Mir)
What is electropermeabilization?

- **Electropchemotherapy** is much more efficient than the usual chemotherapy alone.
- The treatment of a tumor is local and thus has much less side effects.
- The patient has to undergo a lesser number of treatment.
- Electropermeabilization can be reversible or not: the **irreversible** phenomenon can be used for the destruction of cells, and it has also other applications (killing germs, improving extraction of juice from fruits and vegetables).
What is electropermeabilization?

An example of dramatic results may be seen on the chest of this patient (according to Lluis Mir and Julie Gehl)
What is electropermeabilization?

However there are a few drawbacks

- As of today deep tumors cannot be treated, although there are several experiments undertaken around the world

- Due to the high voltage of the electric field (in the range of 5–25 kV/cm) some patients cannot undergo the treatment (pacemakers, anticoagulant therapy)

- The process of the electropermeabilization, or *electroporation*, is not yet well understood, thus the dosage may not be well calibrated
Mathematical questions
Mathematical questions

- Understand, via a mathematical model, the electropermeabilization phenomenon for a cell

- Experiments show that the electropermeabilization is reversible: the permeability of the membrane disappears after a certain time once the electric field is turned off: one would like to estimate the amount of time during which the membrane remains « permeable »

- Understand how to go from one cell to a cluster of cells which makeup a tumor

- Set up a model for electropermeabilization so that the electrochemotherapy can be coupled with models in which the growth of tumors can be predicted
Mathematical questions

▶ Use a mathematical model for the electropermeabilization in order to optimize the placement of the electrodes.

▶ Once an affordable model is set up, and the « direct » problem is solved, study the « inverse » problem, that is the determination of characteristics of the cell membrane.
A molecular dynamics approach
A molecular dynamics approach

One considers $N$ molecules indexed by $j$, located at $x_j(t)$, having mass $m_j$ and charge $q_j$ for $1 \leq j \leq N$

Then one writes the equilibrium of the forces acting on each molecule, assuming that there is an electric field $\mathbf{E}$:

$$m_i \frac{d^2 x_i}{dt^2} = \sum_{j \neq i} \frac{q_i q_j}{|x_i - x_j|^3} (x_i - x_j) + q_i \mathbf{E}(t)$$

...and one solves numerically these equations...
A molecular dynamics approach

For instance here is what one may see, according to D.P. Tieleman 2004

Figure: The Tieleman results. Applied fields at $t = 0$: $0.5\text{V}/\text{nm}$.

../films/Tieleman
film.mpg
A model for the membrane
A model for the membrane

However one may conjecture that as a matter of fact, what happens is something like

That is first the dipoles (heads) get oriented and then there is a partial collapse of the phospholipids. There is no « pore », but rather a sharp decrease of the thickness, which is enough for the passage of molecules.
A model for the membrane
A model for the membrane

- We denote by $\mathbf{n}$ the normal to the mean surface of the membrane, and by $h_\pm$ the height of the dipole above or below this surface

\[ h_\pm = \pm \mathbf{n} \cdot \ell_\pm + \max(0, p_\pm \cdot \mathbf{n}) \]

- Then $h := h_+ + h_-$ is the thickness of the membrane.

- The dipoles $p_\pm$ tend to rotate when submitted to an electric field.

- We assume that they satisfy a Landau-Lifschitz-Gilbert equation, namely:

\[
\begin{align*}
\frac{\partial p_\pm}{\partial t} &= \alpha_1 p_\pm \times (\nabla v_{\Gamma_\pm} + G_\pm) - \alpha_2 p_\pm \times \frac{\partial p_\pm}{\partial t} \\
p_\pm(0) &= c_\pm G_\pm
\end{align*}
\]
A model for the membrane

- The electric potential $v$ is a harmonic function in the exterior of the cell, as well as in the cell.

- The boundary conditions are

$$\begin{cases}
\sigma_c \frac{\partial v_c}{\partial n} = \sigma_e \frac{\partial v_e}{\partial n} & \text{on } \Gamma \\
\frac{\partial v_e}{\partial t} - \frac{\partial v_c}{\partial t} = \alpha_3 h(t, x) \frac{\partial v_0^c}{\partial n} & \text{on } \Gamma
\end{cases}$$

where $v_0^c$ is a potential obtained by an asymptotic analysis, making the thickness $\delta$ tend to zero. See the domain.

- Existence and uniqueness of the solution is established by reducing the system to a new system written on $\Gamma$ (using Steklov-Poincaré operators).

- Numerical simulations made by Frédéric de Gournay show that there is « permeabilization » in some spots, this behavior is not symmetric, and the membrane returns to its initial position once the electric field is turned off.
Schwan’s model
Schwan’s model

- The cytoplasm is homogeneous and conducting.

- The membrane is homogeneous and very insulating ($\sigma_m \in [10^{-7}, 10^{-5}] \text{ S/m}$, while $\sigma_{\text{copper}} \sim 10^7 \text{ S/m}$, $\sigma_{\text{seawater}} \sim 5 \text{ S/m}$, $\sigma_{\text{air}} \sim 10^{-15} \text{ S/m}$).

\[\Omega_m\]

\[\ell h\]

\[\sigma_i = 1 \text{ S/m}\]
\[\varepsilon_i = 10^2 \varepsilon_0\]

\[\Omega_i\]

\[2\ell \sim 2\mu\text{m}\]

\[\sigma_m \sim 10^{-5} \text{ to } 10^{-7} \text{ S/m}\]
\[\varepsilon_m \sim 10\varepsilon_0\]
\[h \sim 10^{-3} \text{ to } 10^{-2}\]
Schwan’s model

- The electric potential $v$ satisfies a time dependent quasistatic equation

$$\frac{\partial}{\partial t} \text{div}(\varepsilon \nabla v) + \text{div}(\sigma \nabla v) = 0 \quad \text{in } (0, \infty) \times \Omega$$

$$v(0, x) = 0 \quad \text{in } \Omega.$$  

$$v(t, x) = v_{\text{imp}} \quad \text{on } (0, \infty) \times \partial \Omega$$

- Or a quasi-static equation in time harmonic regime: setting $\tilde{\varepsilon} := \varepsilon + i\sigma/\omega$, then $v$ satisfies

$$\begin{cases}
\text{div}(\tilde{\varepsilon} \nabla v) = 0 & \text{in } \Omega \\
v(t, x) = v_{\text{imp}} & \text{on } \partial \Omega
\end{cases}$$

(2)
Schwan’s model

The different subdomains involved are as follows:
A static model
After obtaining an asymptotic model as $h \to 0$ we end up with a nonlinear PDE on the domain
A static model

- We consider a positive function \( \lambda \mapsto S_m(\lambda) \), such that for some \( \delta > 0 \),

\[
S_m(\lambda) \sim S_L \quad \text{for} \quad |\lambda| < (1 - \delta)V_*, \quad S_m(\lambda) \sim S_{ep} \quad \text{for} \quad |\lambda| > (1 + \delta)V_*
\]

(S is for Siemens, the unit for the electric conductance, which is also 1/Ohm)

- Typically \( S_m(\lambda) := S_L + (S_{irr} - S_L)\beta(\lambda) \), where \( \beta \) is a function such as

\[
\beta(\lambda) := \left[ 1 + \tanh(k_{ep}|\lambda| - V_{rev}) \right]/2
\]

- We assume that the potential \( V \) satisfies (see the domain).

\[
\begin{cases}
\Delta V = 0 & \text{in} \quad \Omega_e \cup \Omega_i \\
[\sigma \partial_n V] = 0 & \text{on} \quad \Gamma \\
S_m([V]_\Gamma) [V]_\Gamma = \sigma_i \partial_n V_{|r_-} & \text{on} \quad \Gamma \\
V = g & \text{on} \quad \partial \Omega.
\end{cases}
\]

with \([V] := V_{|r_+} - V_{|r_-} \).
A static model

In order to write (7.3) as an equation on the boundary $\Gamma$ we introduce the following Steklov-Poincaré operators:

- $\Lambda_i$ is defined on $H^{1/2}(\Gamma)$ when $\Gamma$ is considered to be $\Gamma := \partial \Omega_i$

- $\Lambda_e$ is defined on $H^{1/2}(\Gamma)$ when $\Gamma$ is considered to be $\Gamma := \partial \Omega_e \setminus \partial \Omega$ (see the domain)

- Finally $\Lambda_0$ is defined as a mapping $H^{1/2}(\partial \Omega) \rightarrow H^{-1/2}(\Gamma)$

These operators are (pseudo-differential operators) of order 1 and are given by:

$$
\begin{align*}
\Lambda_i(f) &:= n_i \cdot \nabla v_{i|\Gamma_-}, \quad \text{with } \Delta v_i = 0 \text{ in } \Omega_i, \ v_i = f \text{ on } \Gamma_- \\
\Lambda_e(f) &:= n_e \cdot \nabla v_{e|\Gamma_+}, \quad \text{with } \Delta v_e = 0 \text{ in } \Omega_e, \ v_e = f \text{ on } \Gamma_+, \ v_e = 0 \text{ on } \partial \Omega. \\
\Lambda_0(f) &:= n_e \cdot \nabla v_{e|\Gamma_+}, \quad \text{with } \Delta v_e = 0 \text{ in } \Omega_e, \ v_e = 0 \text{ on } \Gamma_+, \ v_e = f \text{ on } \partial \Omega.
\end{align*}
$$
A static model

The equation (7.3) above can be written as follows:

1. Let \( u_e := V_{|r_+} \) and \( u_i := V_{|r_-} \), then \( u \in H := H^{1/2}(\Gamma) \times H^{1/2}(\Gamma) \) is given by

   \[
   \begin{cases}
   \sigma_e \Lambda_e u_e + S_m (u_e - u_i)(u_e - u_i) = -\sigma_e \Lambda_0 (g), \\
   \sigma_i \Lambda_i u_i - S_m (u_e - u_i)(u_e - u_i) = 0.
   \end{cases}
   \]

2. Upon setting \( F(s) := \int_0^s S_m(z) z dz \) and

   \[
   (7.5) \quad \Lambda u := \begin{pmatrix} \sigma_e \Lambda_e u_e \\ \sigma_i \Lambda_i u_i \end{pmatrix}
   \]

3. One shows that the solution of (7.4) is obtained as the minimum of

   \[
   (7.6) \quad J(u) := \frac{1}{2} \langle \Lambda u, u \rangle + \int_{\Gamma} F(u_e(\tau) - u_i(\tau)) d\tau.
   \]
A dynamic model
A dynamic model

We consider a positive function \((t, \lambda) \mapsto \tilde{S}_m(t, \lambda)\) defined by,

\[
\tilde{S}_m(t, \lambda) := S_L + (S_{\text{irr}} - S_L)X(t, \lambda).
\]

Here, for some \(X_0 \in [0, 1]\) given and \(\beta\) defined in (7.2), \(X(t, \lambda)\) satisfies

\[
\frac{\partial X(t, \lambda)}{\partial t} = \max\left(\frac{\beta(\lambda) - X(t, \lambda)}{\tau_{\text{ep}}}, \frac{\beta(\lambda) - X(t, \lambda)}{\tau_{\text{res}}}\right), \quad X(0, \lambda) = X_0
\]

We assume that the potential \(V\) satisfies (see the domain).

\[
\begin{cases}
\Delta V = 0 & \text{in } (0, T) \times (\Omega_e \cup \Omega_i) \\
[\sigma \partial_n V] = 0 & \text{on } \Gamma \\
C_m \frac{\partial [V]_\Gamma}{\partial t} + \tilde{S}_m(t, [V]_\Gamma) [V]_\Gamma = \sigma_i \partial_n V_{\Gamma-} & \text{on } (0, T) \times \Gamma \\
V = g & \text{on } (0, T) \times \partial \Omega \\
V(0, x) = V_0(x) & \text{in } \Omega_e \cup \Omega_i.
\end{cases}
\]
A dynamic model

We write the above equation as follows:

- First we show that $\Lambda_e + \Lambda_i$ is self-adjoint, positive and invertible. Then we set $B := I + \Lambda_e^{-1} \Lambda_i$, and

  $$v := u_i - u_e := V_{l^-} - V_{l^+}$$

- One checks that

  $$u_i = V_{l^-} = B^{-1}(v - \Lambda_e^{-1} \Lambda_0 g)$$

- And setting

  $$G := \Lambda_i B^{-1} \Lambda_e^{-1} \Lambda_0 g, \quad \varphi := (V_0)_{l^-} - (V_0)_{l^+}$$
A dynamic model

Then $v$ satisfies

$$\begin{cases}
C_m \partial_t v + \Lambda_i B^{-1} v + \tilde{S}_m(t, v) v = G & \text{on } (0, T) \times \Gamma \\
v(0, \cdot) = \varphi(\cdot) & \text{on } \Gamma.
\end{cases}$$

Here $\tilde{S}_m(t, v) = S_L + (S_{ir} - S_L)X(t, v)$ and $X$ satisfies

$$\begin{cases}
\frac{\partial X}{\partial t} = \max \left( \frac{\beta(v) - X}{\tau_{ep}} ; \frac{\beta(v) - X}{\tau_{res}} \right) \\
X(0, v) = X_0 \in [0, 1].
\end{cases}$$

One proves that these equations have a unique solution.
A dynamic model

- Other phenomena can also be taken into account, upon setting

\[
\begin{align*}
\tilde{S}_m(t,v) &= S_L + S_1X_1(t,v) + S_2X_2(t,v) \\
\tilde{P}_m(t,v) &= P_L + P_1X_1(t,v) + P_2X_2(t,v)
\end{align*}
\]

- Here \( \tilde{P}_m \) is the permeability of the membrane, and \( X := (X_1, X_2) \) satisfies an equation of the type

\[
\partial_t X = F(v(t, X), X).
\]

- Some numerical simulations by Michael Leguèbe are obtained, and may be compared quite satisfactorily with the experiments done by Aude Silve.