ELECTROPERMEABILIZATION MODELING AT CELL SCALE

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A new electropermeabilization (EP) model inspired from the static model of Ivorra *et al.* [1] is derived in order to monitor the behaviour of both **transmembrane potential (TMP)** and **surfacic conductivity** of a cell, when submitted to an electric pulse. This phenomenological model runs with fewer parameters than the probably most achieved ones, proposed by Neu and Krassowska [2], thus **facilitating the fitting** to experiments. We use a numerical scheme adapted from a method by Cisternino and Weynans [3]. It is based on an accurate discretization of the fluxes at an interface, through the use of additional interface unknowns. The diffusion of therapeutic agents in the cell such as bleomycin is eventually presented in order to link numerical simulations with the experiments.

MODELING PRINCIPLE

Extracellular medium O_e and cytoplasm O_i separated by the cell membrane Γ are considered as homogeneous isotropic media, with the respective conductivities σ_e and σ_i.

S_m and C_m designate the membrane's surfacic conductivity and capacitance.

When the pulse g is applied to the cell, the electric potential u verifies

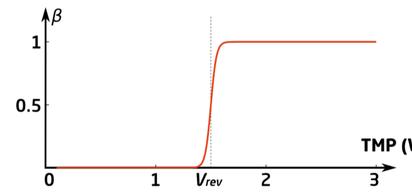
$$\begin{cases} \nabla.(\sigma\nabla u) = 0 & \text{in } \mathcal{O}_e,\ \mathcal{O}_i \\ [\sigma\partial_{\boldsymbol{n}} u] = 0 & \text{on } \Gamma \\ C_m\partial_t[u] + S_m[u] = \sigma_i\partial_{\boldsymbol{n}} u_i & \text{on } \Gamma \\ u = g & \text{on } \partial\Omega \end{cases}$$

In the model of Neu and Krassowska the EP is described through a highly non-linear current term, which depends on a large number of parameters. Here we take the EP into account through the membrane conductivity $S_m = S_L + x(S_{ir}-S_L)$, $x \in [0,1]$. S_L is the membrane conductivity when no pulse is applied, whereas S_{ir} is its value when fully electroporated.

x follows a sliding door model :

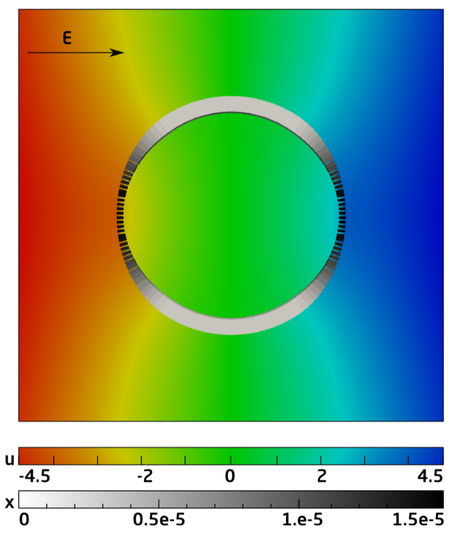
$$\begin{cases} \partial_t x = \max\left(\dfrac{\beta([u]) - x}{\tau_{ep}}, \dfrac{\beta([u]) - x}{\tau_{res}}\right) \\ x(0) = 0 \end{cases}$$

$\beta = \mathcal{H}(V_{rev}, k_{ep})$ is a regularized Heaviside function around a threshold TMP V_{rev}, which has been highlighted by all the membrane models, and above which electropermeabilization occurs.
When $\beta > x$, the electric pulse is sufficiently high to enlarge the permeabilized region, with a dynamic τ_{ep}. On the contrary, when $\beta < x$, the membrane tries to reseal with a characteristic time τ_{res} which is much larger than τ_{ep}.



PRELIMINARY RESULTS

Electric potential u and electropermeabilization coefficient x in a single cell, under the influence of a uniform 400 V/cm field.

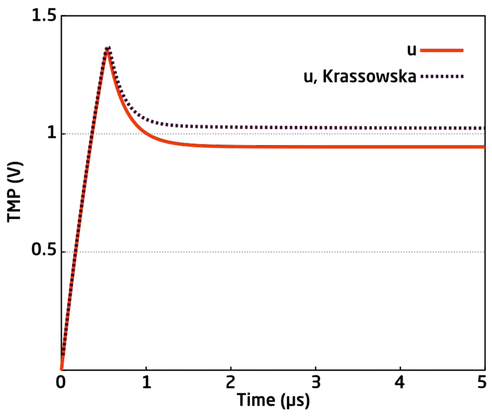


Physiological parameters mainly come from Krassowska *et al.* papers. The specific parameters of our model were first set to fit with the results of [2]

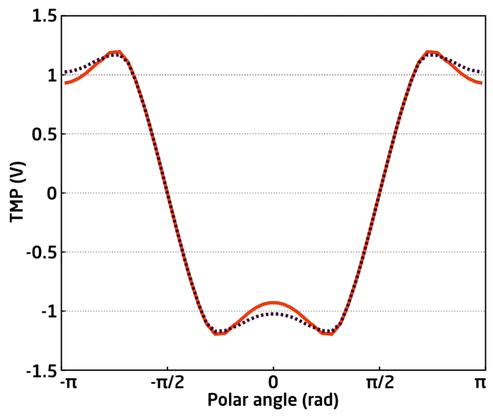
Variable	Value
Physiology	
External conductivity σ_e	5 S/m
Cytoplasm conductivity σ_i	0.455 S/m
Capacitance C_m	9.5 10^{-3} F/m^2
Surfacic membrane conductivity S_L	1.9 S/m^2
Model	
EP threshold V_{ep}	1.5 V
EP switch speed k_{ep}	40 V^{-1}
EP speed τ_{ep}	10^{-6} s^{-1}
Reseal time τ_{res}	10^{-2} s^{-1}
EPd membrane conductivity S_{ir}	0.25 10^9 S/m^2
Simulation	
Cell radius	50 µm
Membrane thickness	5 nm
Domain size	200 µm
Grid points per side	100
Initial time pace Δt	20 ns
Measuring time T_f	100 µs

COMPARISON WITH KRASSOWSKA MODEL

TMP [u] at the rear of the cell (θ=0)

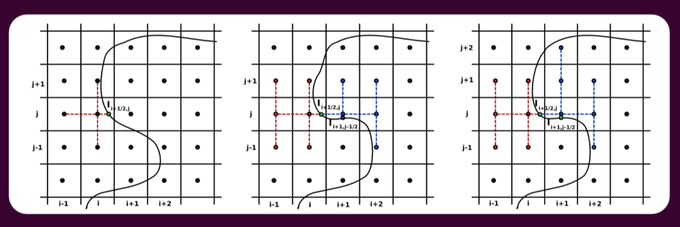


TMP [u] along the perimeter

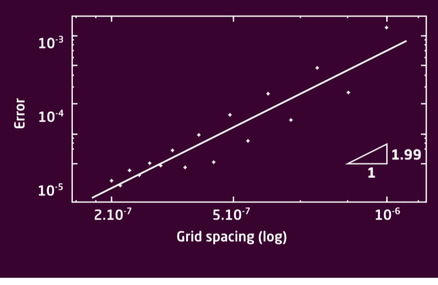


SECOND ORDER FINITE DIFFERENCES CARTESIAN METHOD

The method used to accurately discretize the fluxes and the jump conditions on Γ is based on the insertion of additional unknowns on each side of the interface. Points neighbouring the interface are treated with specific stencils to approximate the Laplacian (see below, left panel). Fluxes $\partial_{\boldsymbol{n}} u = \nabla u.\boldsymbol{n}$ on the interface points are discretized at the second order in each direction (middle panel). If the obtained stencil contains several interface points instabilities may appear. To avoid this problem transverse stencils are shifted as shown on the right panel.



It is possible to derive an analytic solution of the linear problem (S_m=S_L), when studying a circular cell in a field of the form $g = E r\cos\theta$. Compared to this solution, our scheme achieves a second order convergence. The order is lower when EP is taken into account.



Evolution of the whole system is obtained by solving at each time iteration :

$$\begin{cases} \Delta u^{n+1} = 0 \\ \left[\sigma\partial_{\boldsymbol{n}} u^{n+1}\right] = 0 \\ \dfrac{C_m}{\Delta t}\left[u^{n+1}\right] - \sigma_i\partial_{\boldsymbol{n}} u_i^{n+1} = \dfrac{C_m}{\Delta t}[u^n] - S_m(x^n, [u^n])\,[u^n] \end{cases}$$

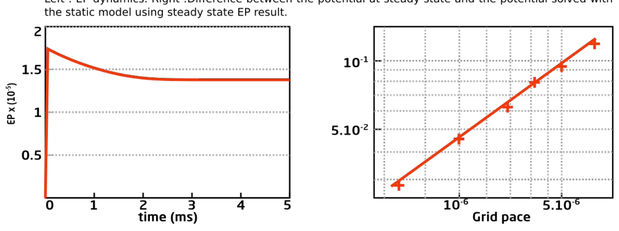
The EP coefficient x is updated with the standard RK4 scheme. Since the dynamics of the EP and of the membrane resealing occur at very different time scales, an adaptative time pace is chosen, so as the implicit part of the iteration matrix has its spectral radius lower than 1.

$$\Delta t < 2\frac{C_m}{\max(S_m)}$$

DYNAMIC VS STATIC MODEL

SImulations were run up to several milliseconds - the order of τ_{res} - in order to reach the steady state. The static equation, in which the dielectric term C_m is neglected, is also solved with the same parameters. Results show TMP profiles which are close to the steady state ones. One must note that estimations on EP given by a static model are only pertinent when millisecond pulses are applied.

Left : EP dynamics. Right :Difference between the potential at steady state and the potential solved with the static model using steady state EP result.



BLEOMYCIN DIFFUSION

In order to make the link between the numerical results and the survival rates given by the experiments, a 0D drug model is set up. Assuming that the flow of molecules through the membrane is proportional to the EP coefficient x, the bleomycin concentration B follows the ODE

$$\partial_t B = B_{\text{ext}} \int_\Gamma D x(t,l)\ \mathrm{d}l,\ B(0) = 0$$

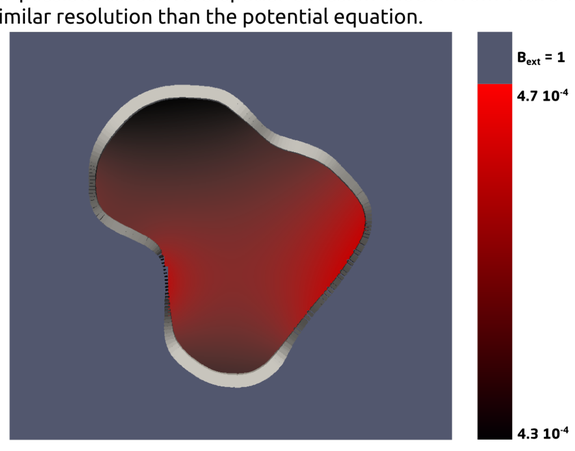
where D is a parameter which has to be calibrated with the experiments.

It is known that passed a given intracellular concentration of bleomycin, apoptosis is very likely to happen. Moreover a too important value of electroporation considerably affects the membrane's structure. We propose the following cell survival probability:

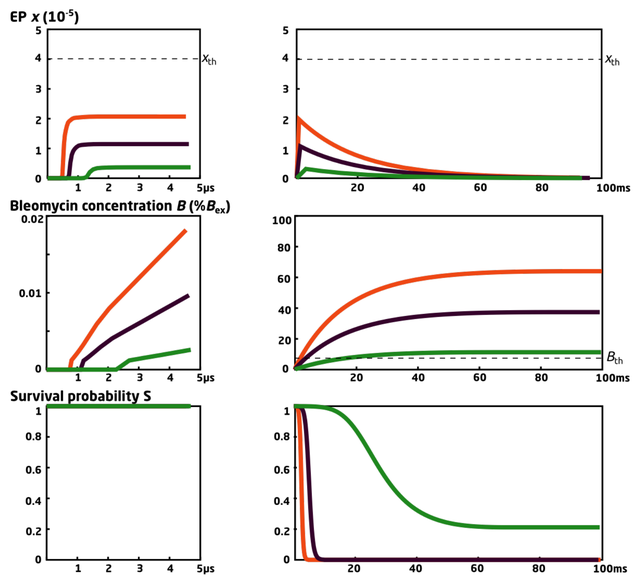
$$S(x,B) = (1 - \mathcal{H}_{B_{th}}(B))(1 - \mathcal{H}_{x_{th}}(\bar{x}))$$

meaning that the concentration B must not exceed the threshold B_{th} and the same for the average of x on Γ with x_{th}. Several simulations were simultaneously run on cells with different characteristic sizes to compute survival rate of a distributed cell population.

It is also possible to derive a spatial diffusion model from Fick's law, with a similar resolution than the potential equation.

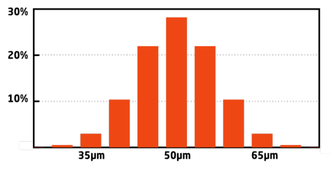


Bleomycin diffusion right after the pulse application at t=10 µs



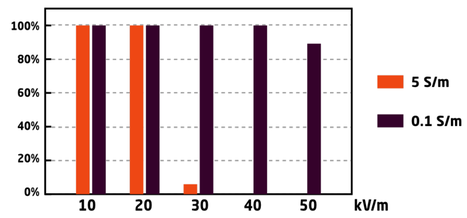
Results of a single cell simulation on EP rate x, bleomycin concentration B and survival probability S for a 100 µs pulse of various magnitudes.

Cell population distribution for which simulations were run in parallel.

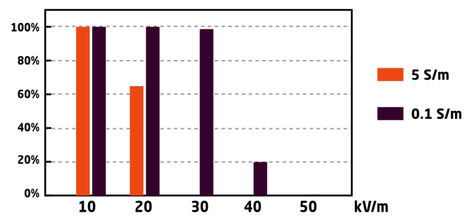


Survival rates after application of 1 pulse followed by 100 ms of relaxation, for two solutions of different conductivities σ_e.

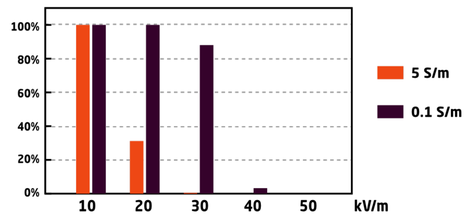
1 pulse, 100 µs.



3 pulses, 100µs, 1 ms between each.



8 pulses, 100µs, 1 ms between each



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[2] DeBruin K. and Krassowska W.. Modeling electroporation in a single cell. I. Effects of filed strength and rest potential. Biophysical Journal. 1999.
[3] Cisternino M. and Weynans L. A parallel second order method for elliptic interface problems. CiCP (12,5). 2012.

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