Interpulsed multifrequency electrical impedance measurements during electroporation of adherent differentiated myotubes

Tomás García-Sánchez [1], Antoine Azan [2,3,4], Isabelle Leray [2,3,4],
Javier Rosell-Ferrer [1], Ramón Bragos [1], and Lluis M. Mir [2,3,4]

(1) Department of Electronic Engineering, Univ; Politècnica de Catalunya, SPAIN
(2) CNRS, UMR 8203, FRANCE
(3) Univ. Paris Sud, UMR 8203, FRANCE
(4) Gustave Roussy, UMR 8203, FRANCE

Plasma membrane of living cells constitutes the barrier between the intracellular and extracellular media and regulates the transport of chemical species from or into the cell cytoplasm. Electroporation, also called electropermeabilization, is a phenomenon occurring when cell membranes are exposed to high electric field pulses. When the parameters of such electric field are appropriate, a transient state of permeability to molecular species is generated. The technique is currently used as a tool to deliver membrane impermeable molecules into the cells both in vitro and in vivo with its main interest focused in electrochemotherapy of tumors and nucleic acids electrotransfer for gene therapy or DNA vaccination.

Monitoring in real-time the permeability state of the cell membranes during electroporation treatment represents an encouraging challenge with applications both in the theoretical and practical scenarios. On the one hand, it is interesting to provide information of the membrane dynamics to better understand the underlying mechanisms that govern the process. On the other hand, this information could be valuable in the optimization of electroporation treatments.

In this study electrical impedance spectroscopy measurements are performed during elec-troporation of differentiated myotubes monolayers. The time resolution of the system (1 spectrum/ms) enables to acquire 860 full spectra during the interpulse time window between consecutive pulses of a classical electroporation treatment (8 pulses, 100 µs, 1 Hz) (see Fig. 1a). Additionally, the characteristics of the custom microelectrode assembly designed allow performing the experiments directly in situ and with no contact with cells in standard 24 multi-well plates (see Fig. 1b).

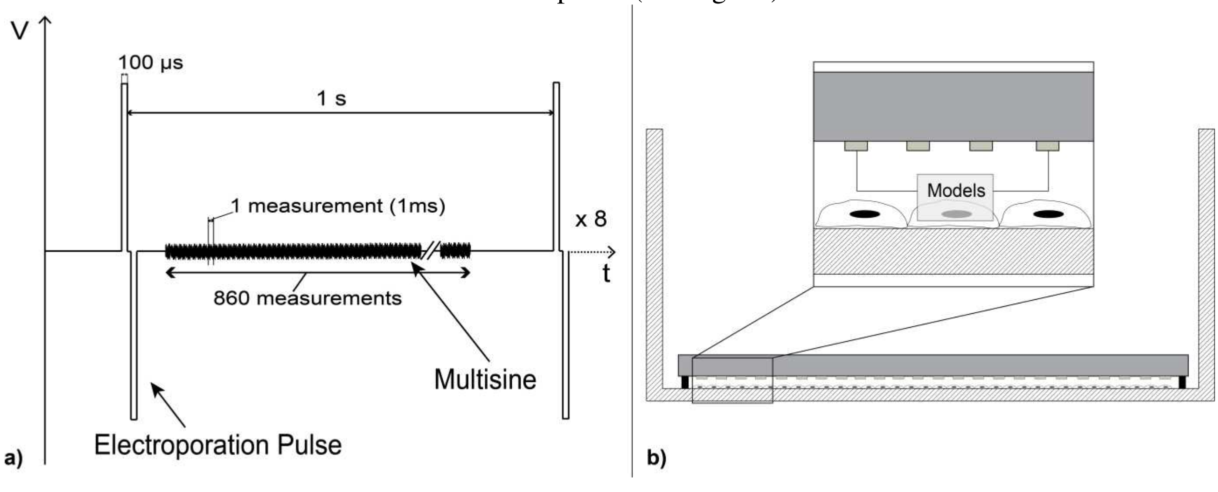

Fig. 1 a) Schematic representation of the measuring strategy during electroporation. b) The concept of the microelectrode assembly used.

The multifrequency information, analyzed with the Cole model, reveals two different membrane dynamics in agreement with the existence of two populations of pores (short-lived and long-lived pores). The time constants of the short-lived pores dynamics are studied for different electric field intensities. The analysis shows differences between the lowest electric field condition and the other two suggesting that different mechanisms, maybe related with the reversibility of the process, are activated. Thanks to the multifrequency information, the system is able to measure simultaneously the conductivity variations due to ion diffusion during electroporation. Finally, in order to reinforce the physical interpretation of the results, a complementary electrical equivalent model is used.