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Department of Electrical Engineering and Power Electronics

Principle of Electroporation for medical purposes used for Jett Plasma for Her from COMPEX

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Synthetic summary

- What is electroporation?
- Types of electroporation
- Mode of action
- Electroporation process applied to the treatment of vulvo vaginal laxity
- Summary

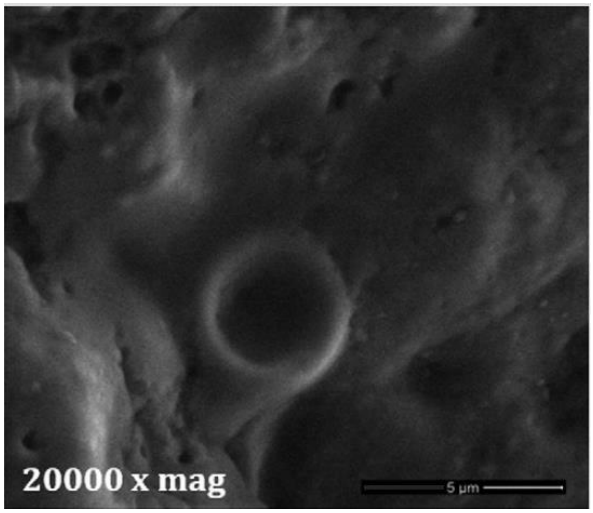
What is electroporation?

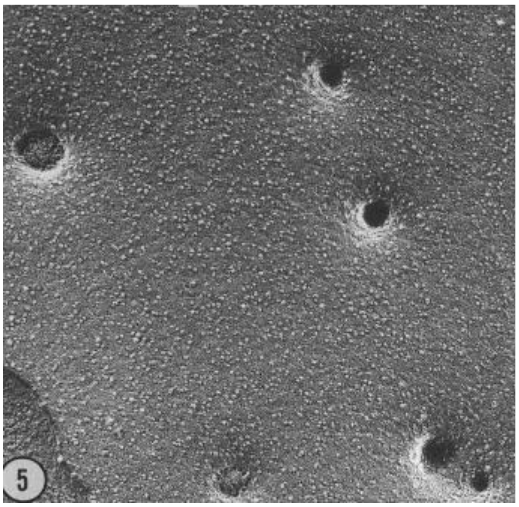
Electroporation is a process in which brief electrical pulses create transient pores in the plasma membrane that allow nucleic acids to enter the cell cytoplasm. (1)

It increases the permeability of the cell membrane after this short high voltage pulse applied to the medium.

- **Electroporation can be transient** (2) (reversible in the case of Jett Plasma) for an exchange between the medium and the cell, without altering the cell or its membrane with a return to the initial state after the pulse.
  - ✓ Electroporation with addition of molecules on the treated area > targeted treatments
  - ✓ Electroporation without addition of molecules > exchange with assimilation H_2O + nutrients
- **Electroporation** may be irreversible (4) for other applications - possible programmed cell destruction - apoptosis (**not used with Jett Plasma**).

Examples of transient nanopores during the pulse (3)



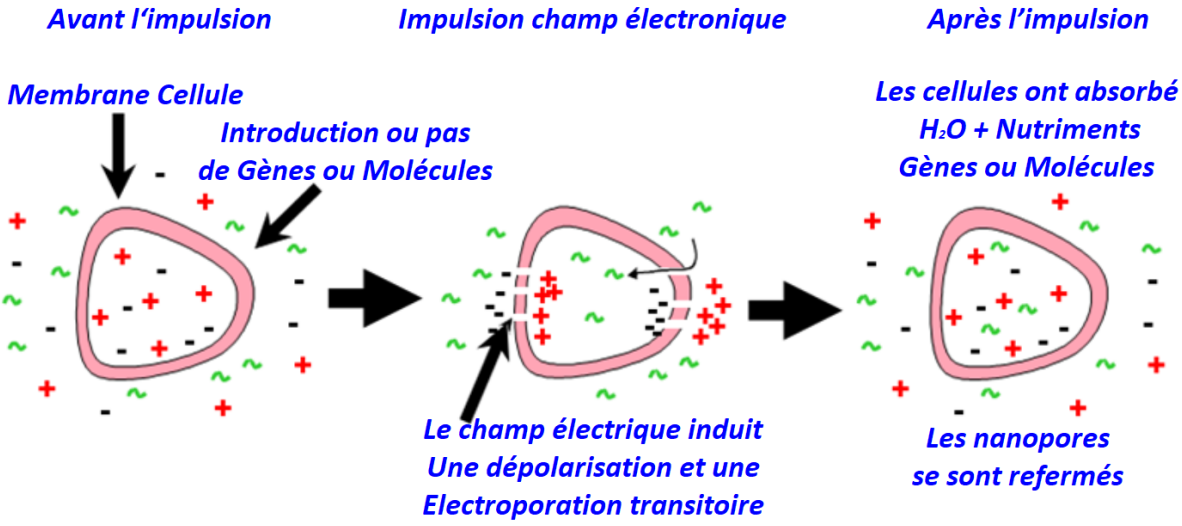


Types of electroporation and mode of action

Reversible Electroporation (2) (6)
(How Jett Plasma for Her works)

- *Transient nanopores, uptake into cells:*
  - *H2O,*
  - *Proteins,*
  - *Medications,*
  - *Genes, etc.*

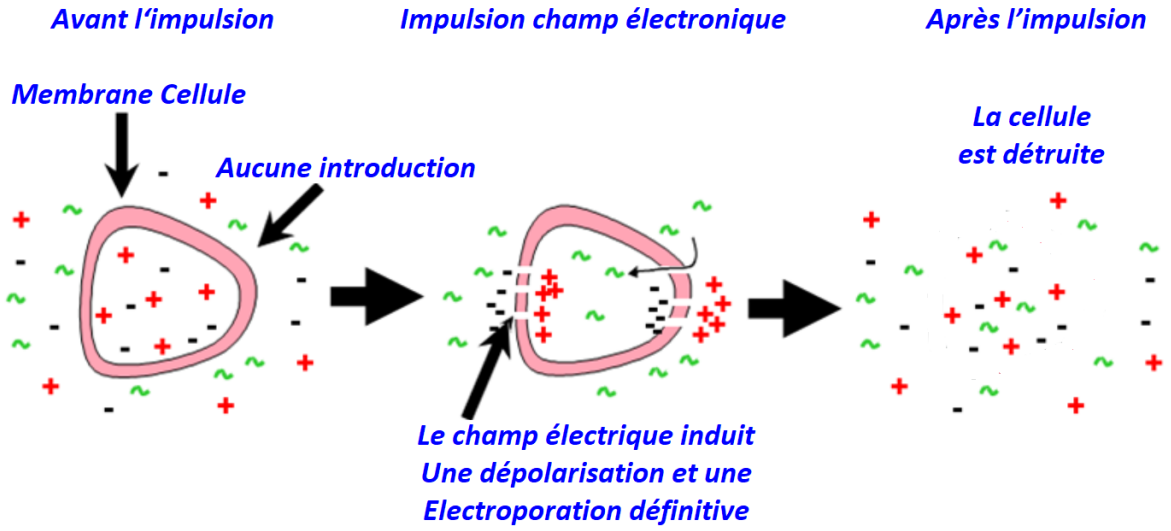
$$\left|\vec{E}_{rev}\right| \leq \left|\vec{E}\right| \leq \left|\vec{E}_{irrev}\right|$$



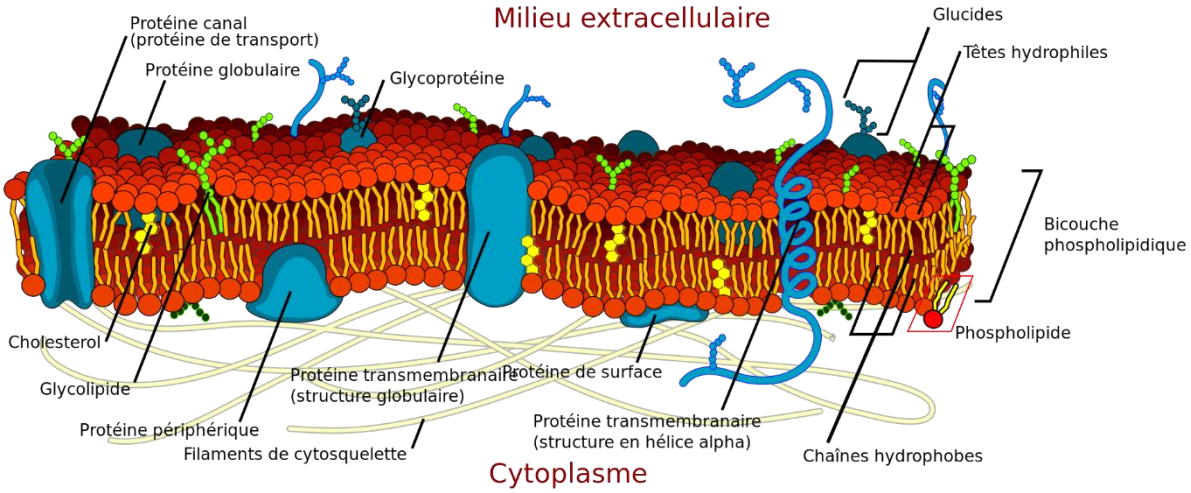
Irreversible Electroporation (4) (5)
(Only For information, does not concern the Jett Plasma for Her)

- *Permanent nanopores, absorption into cells:*
  - *Permanent pores*
  - *Sterilization,*
  - *Tissue removal,*

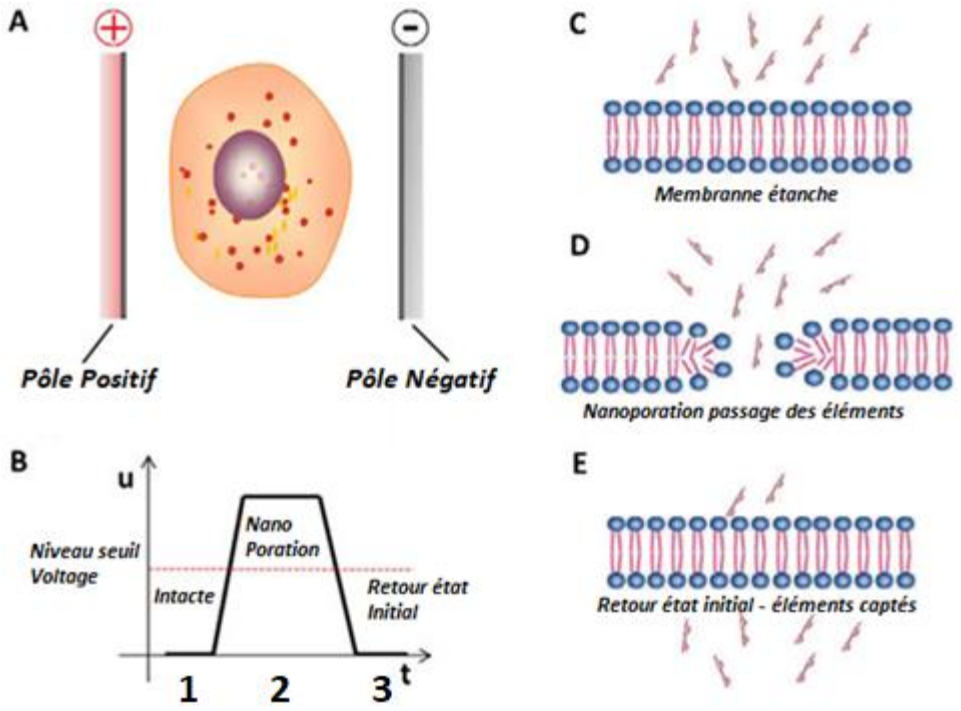
$$\left|\vec{E}_{irrev}\right| \leq \left|\vec{E}\right| \leq \left|\vec{E}_{therm}\right|$$



Fluid mosaic of a cell membrane, biological model designed by Seymour Jonathan Singer et Garth L. Nicolsonen 1972,



Example of electroporation: gene therapy carried out in the reversible poration phase; this diagram makes it possible to understand how the nanopore is formed on the cell membrane.



← Time 1 - Before pulse

← Time 2 - During pulse

← Time 3 – After pulse

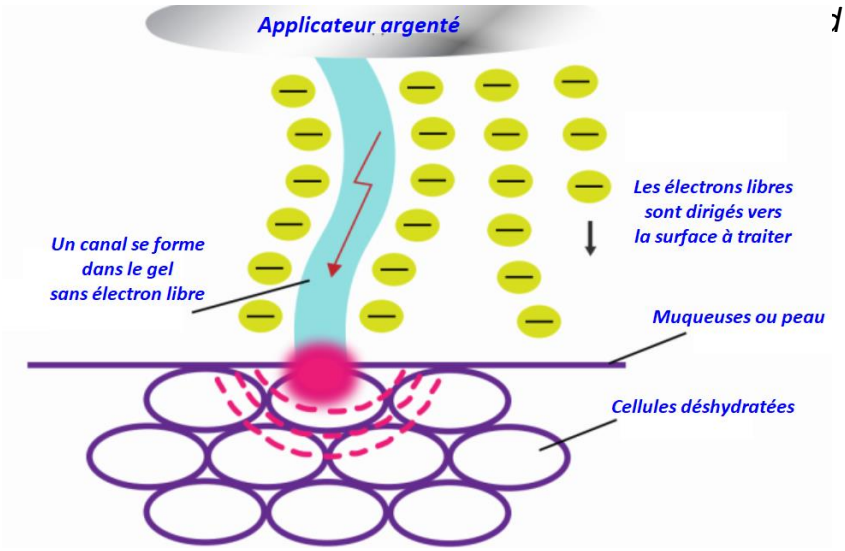
...a common physical tool electric field for altering cell membrane permeability is called electroporation. It was first mentioned in 1982 by Neumann who used electroporation to transfect mouse lyoma cells. The permeability of the cell membrane has a strong correlation with the intensity of the external electric field. Depending on the exposure time and electric field strength, the electroporation process is divided into four continuous phases including no detectable poration, reversible poration, non-thermal irreversible poration, and thermal irreversible poration, respectively (Yarmush et al. , 2014; Megli & Kotnik, 2015). Gene therapy is performed in the reversible poration phase (Miklavcic et al., 2014; Wagstaff et al., 2016), and the principle is shown in Figure 1. A living cell is exposed in the external impulse electric field (Figure 1A). When the strength of the external electric field exceeds the threshold voltage, the transient pore forms in the cell membrane and exogenous nucleic acids are delivered into the cell (Figure 1D). Then, resealing of the cell membrane occurs over a range of minutes (Figure 1E) after the external field strength drops to the threshold...

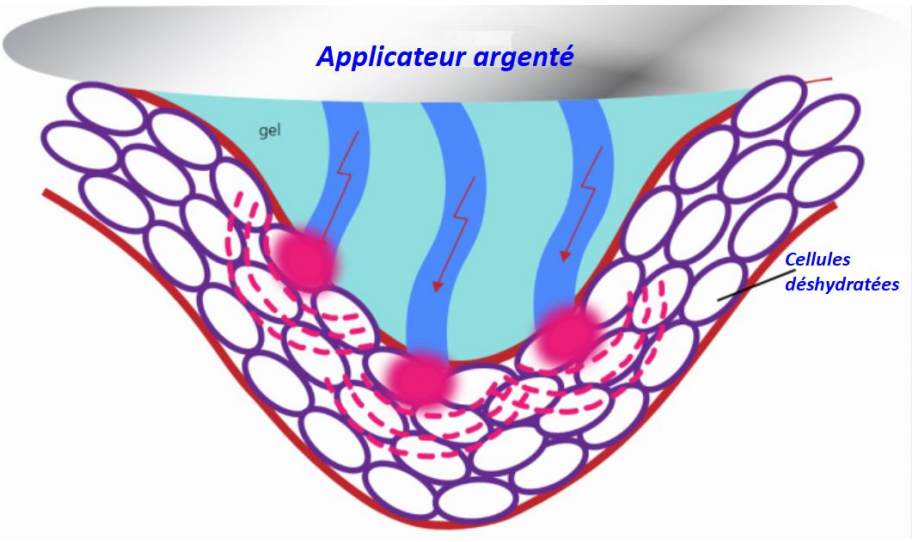
The electroporation process used with Jett Plasma For Her

A film of neutral gel is applied to the surface to be treated.

- The probe is pressed against the surface

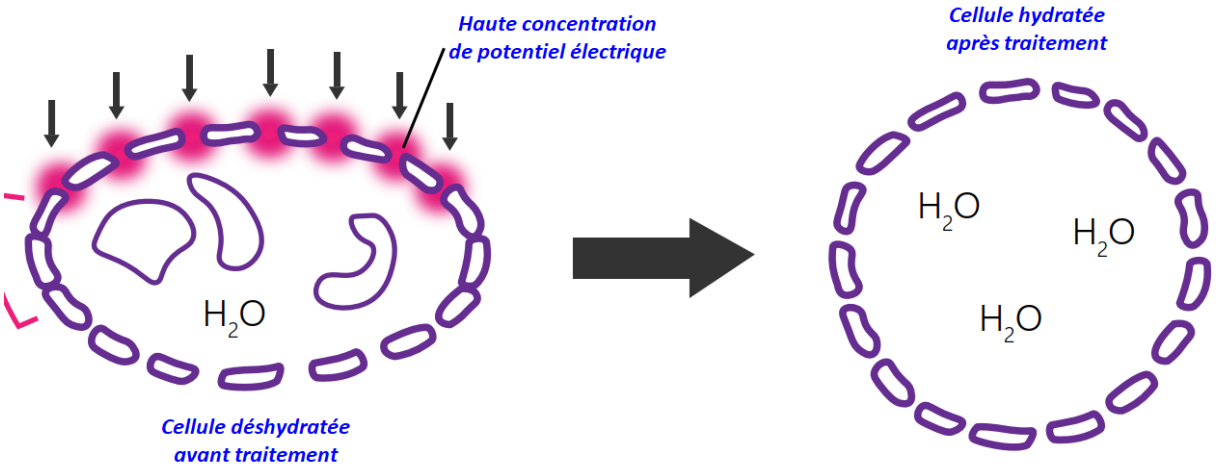
- The energy is delivered continuously during the treatment

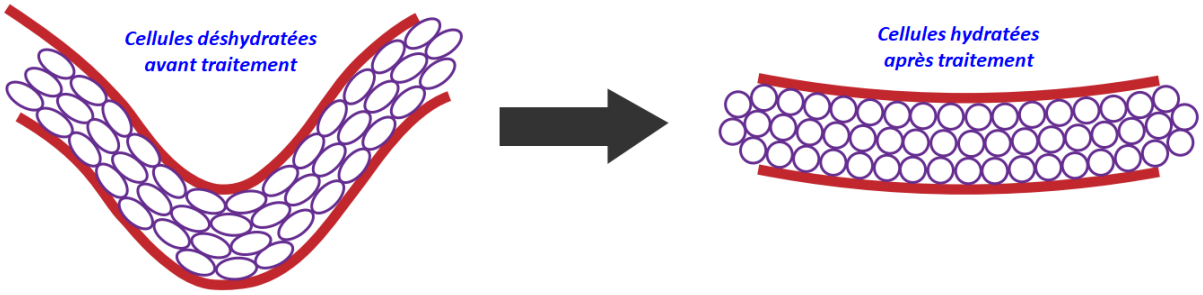




Only the cells next to the plate are treated

Block diagram of cell evolution before and after treatment





Simplified electroporation process used with Jett Plasma For Her

Summary of actions and effects:

Applicator + gel
Polarized electric field (DC)
Micro-discharges through the gel into the tissue
Creation of nanopores on cell membranes
Water, carbohydrates and nutrients circulate from the extracellular volume to the cells

- Painless treatment
- Rejuvenation and revitalization of cells
- Reduction of inflammation,
- The cells become spherical again
- Their natural function is restored

Resume

Reversible electroporation (2) (6) versus irreversible (4) (5)

Reversible electroporation (2) (6) is a safe and effective means of tissue restoration for many indications in different specialties, in gynecology it can treat the following indications:

- Vulvovaginal laxity
- Loss of elasticity and sensitivity
- Incontinence
- Sexual discomfort due to vaginal dryness
- Insufficient vaginal lubrication
- Burning and itching
- Softening of scars
- Remodeling of the vulva and labia

Irreversible electroporation (4) (5) is a solution for targeted tissue removal.
This option is not available on the Jett Plasma for Her.

REFERENCES:

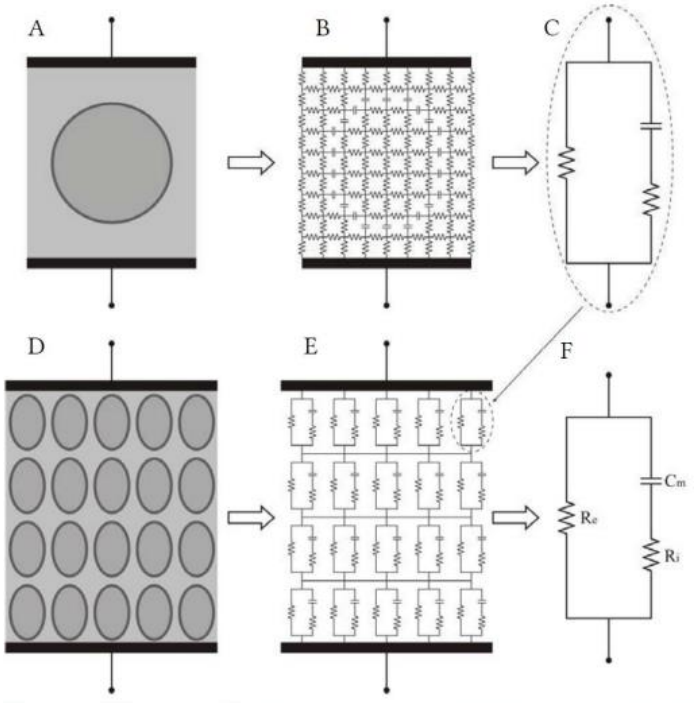
1.) Priti Kumar, Arvindhan Nagarajan, Pradeep D Uchil Electroporation - Cold Spring Harb Protoc-2019-Kumar-pdb.top096271
2.) CHANG, Donald C. a Thomas S. REESE. Changes in membrane structure induced by electroporation as revealed by rapid-freezing electron microscopy, Biophysical Society. 1980, 58, 1-12
3.) Gintautas Saulis[1], Rita Saulė Biochim Biophys Acta . 2012 Déc;1818(12):3032-9. doi: 10.1016/j.bbamem.2012.06.018.EPUB 2012 Juil 2.
4.) JOURABCHI, Natanel, Kourosh BEROUKHIM, Bashir A. TAFTI, Stephen T KEE a Edward W. LEE. Irreversible electroporation (NanoKnife) in canc er treatment. Gastrointestinal Intervention. 2014, 3(1), 8-18.
5.) accelerating intelligence [online]. KurzweiAlNetwork, 2000 [cit. 2017-04-12]. Available at: http://www.kurzweilai.net/electrical-pulse-treatment-pokes-holes-to-treat-tumors
6.) Electroporation. BTX – the expert [online]. USA: BTX [cit. 2017-04-12]. Available at: http://www.btxonline.com/technical-resources/faq.html

Others similar REFERENCES:

1.) Hamilton, W. A., and A. J. H. Sale. 1967. Effects of high electric fieldson microorganisms. II. Mechanisms of action of the lethal effect. Biochim. Biophys. Acta.148:789–800.
2.) Neumann, E., S. Kakorin, and K. Toensing. 1999. Fundamentals ofelectroporative delivery of drugs and genes. Bio electrochem. Bioenerg.48:3–16.
3.) Weaver, J. C., and Y. A. Chizmadzhev. 1996. Theory of electropor-ation: a review.Bioelectrochem. Bioenerg.4:135–160
4.) Hofmann, G. A., S. B. Dev, G. S. Nanda, and D. Rabussay. 1999.Electroporation therapy of solid tumors.Crit. Rev. Ther. Drug CarrierSyst.16:523–569
5.) Widera, G., M. Austin, D. Rabussay, C. Goldbeck, S. W. Barnett,M. C. Chen, L. Leung, G. R. Otten, K. Thudium, M. J. Selby, and J. B.Ulmer. 2000. Increased DNA vaccine delivery and immunogenicity byelectroporation in vivo.J. Immunol.164:4635–4640.
6.) Dev, S. B., D. P. Rabussay, G. Widera, and G. A. Hofmann. 2000.Medical applications of electroporation.IEEE Transactions on PlasmaScience.28:206–223.
7.) Lucas, M. L., and R. Heller. 2003. IL-12 gene therapy using anelectrically mediated nonviral approach reduces metastatic growth ofmelanoma.DNA Cell Biol.22:755–763.
8.) Chen, N., K. H. Schoenbach, J. F. Kolb, S. R. James, A. L. Garner, J.Yang, R. P. Joshi, and S. J. Beebe. 2004. Leukemic cell intracellularresponses to nanosecond electric fields.Biochem. Biophys. Res.Commun.317:421–427.
9.) Hu, Q., S. Viswanadham, R. P. Joshi, K. H. Schoenbach, S. J. Beebe,and P. F. Blackmore. 2005. Simulations of transient membranebehavior in cells subjected to a high-intensity, ultrashort electric pulse.Phys. Rev. E. Stat. Nonlin. Soft. Matter Phys.71:031914.
10.) Schultheiss, C., H. Bluhm, H.-G. Mayer, M. Kern, T. Michelberger,and G. Witte. 2002. Processing of sugarbeets with pulsed-electricfields.IEEE Transactions on Plasma Science.30:1547–1551.
11.) Schoenbach, K. H., R. P. Joshi, R. H. Stark, F. C. Dobbs, and S. J.Beebe. 2000. Bacterial decontamination of liquids with pulsed electricfields.IEEE Transactions on Dielectrics and Electrical Insulation.7:637–645.
12.) Choenbach, K. H., S. J. Beebe, and E. S. Buescher. 2001. Intracellulareffect of ultrashort electrical pulses.Bioelectromagnetics.22:440–448.
13.) Vernier, P. T., Y. H. Sun, L. Marcu, C. M. Craft, and M. A. Gundersen.2004. Nanosecond pulsed electric fields perturb membrane phospho-lipids in T lymphoblasts.FEBS Lett.572:103–108.
14.) Buescher, E. S., and K. H. Schoenbach. 2003. Effects of submicro-second, high intensity pulsed electric fields on living cells-intracellularelectromanipulation.IEEE Transactions on Dielectrics and ElectricalInsulation.10:788–794.
15.) White, J. A., P. F. Blackmore, K. H. Schoenbach, and S. J. Beebe.2004. Stimulation of capacitative calcium entry in HL-60 cellsby nanosecond pulsed electric fields.J. Biol. Chem.279:22964–22972.
16.) Beebe, S. J., P. M. Fox, L. J. Rec, K. Somers, R. H. Stark, and K. H.Schoenbach. 2002. Nanosecond pulsed electric field (nsPEF) effects oncells and tissues: apoptosis induction and tumor growth inhibition.IEEE Transactions on Plasma Science.30:286–292.
17.) Beebe, S. J., P. M. Fox, L. J. Rec, E. L. Willis, and K. H. Schoenbach.2003. Nanosecond, high-intensity pulsed electric fields induce apopto-sis in human cells.FASEB J.17:1493–1495.
18.) Schoenbach, K. H., R. P. Joshi, J. F. Kolb, N. Y. Chen, M. Stacey,P. F. Blackmore, E. S. Buescher, and S. J. Beebe. 2004. Ultrashortelectrical pulses open a new gateway into biological cells.Proc. IEEE.92:1122–1137.
19.) Kinosita, K., Jr., I. Ashikawa, N. Saita, H. Yoshimura, H. Itoh, K.Nagayama, and A. Ikegami. 1988. Electroporation of cell membranevisualized under a pulsed-laser fluorescence microscope.Biophys. J.53:1015–1019.
20.) Hubener, G., A. Lambacher, and P. Fromherz. 2003. Anellatedhemicyanine dyes with large symmetrical solvatochromism of absorp-tion and fluorescence.J. Phys. Chem. B.107:7896–7902.
21.) Kuhn, B., and P. Fromherz. 2003. Anellated hemicyanine dyes in aneuron membrane: molecular Stark effect and optical voltage record-ing.J. Phys. Chem. B.107:7903–7913.
22.) Loew, L. M., G. W. Bonneville, and J. Surow. 1978. Charge shift opticalprobes of membrane-potential: theory.Biochemistry.17:4065–4071.
23.) Loomis-Husselbee, J. W., P. J. Cullen, R. F. Irvine, and A. P. Dawson.1991. Electroporation can cause artifacts due to solubilization of cations from the electrode plates. Biochem. J.277:883–885.
24.) Tomov, T., and I. Tsoneva. 2000. Are stainless steel electrodes inert Biochemistry.51:207–209.

25.) Kolb, J. F., S. Kono, and K. H. Schoenbach. 2005. Nanosecond pulsed electric field generators for the study of subcellular effects.Bioelectromagnetics.27:172–187.
26.) Xu, C., W. Zipfel, J. B. Shear, R. M. Williams, and W. W. Webb. 1996.Multiphoton fluorescence excitation: new spectral windows for biological nonlinear microscopy. Proc. Natl. Acad. Sci. USA.93:10763–10768.
27.) Kuhn, B., P. Fromherz, and W. Denk. 2004. High sensitivity of Stark-shift voltage-sensing dyes by one- or two-photon excitation near thered spectral edge. Biophys. J.87:631–639.
28.) Sarkadi, B., A. Tordai, and G. Gardos. 1990. Membrane depolarizationselectively inhibits receptor-operated calcium channels in human T(Jurkat) lymphoblasts. Biochim. Biophys. Acta.1027:130–140.
29.) Fischer, J. K., D. M. von Bruning, and H. Labhart. 1976. Light-modulation by electrochromism.Appl. Opt.15:2812–2816.
30.) Stewart, D. A., T. R. Gowrishankar, and J. C. Weaver. 2004. Transportlattice approach to describing cell electroporation: use of a local asymptotic model. IEEE Transactions on Plasma Science.30:1696–1708.
31.) Loew, L. M. 1999. Potentiometric membrane dyes and imaging membrane potential in single cells. In Fluorescent and Luminescent Probes for Biological Activity. E. Mason, editor. Academic Press.New York. 210–221.
32.) Tekle, E., R. D. Astumian, and P. B. Chock. 1990. Electro-permeabilization of cell membranes: effect of the resting membranepotential. Biochem. Biophys. Res. Commun.172:282–287.
33.) Mehrle, W., R. Hampp, and U. Zimmermann. 1989. Electric pulse induced membrane permeabilization. Spatial orientation and kinetics ofsolute efflux in freely suspended and dielectrophoretically aligned plantmesophyll protoplasts. Biochim. Biophys. Acta.978:267–275.
34.) Tekle, E., R. D. Astumian, and P. B. Chock. 1994. Selective andasymmetric molecular transport across electroporated cell membranes. Proc. Natl. Acad. Sci. USA.91:11512–11516.
35.) Tekle, E., R. D. Astumian, W. A. Friauf, and P. B. Chock. 2001.Asymmetric pore distribution and loss of membrane lipid in electroporated DOPC vesicles. Biophys. J.81:960–968.
36.) Siwy, Z., and A. Fulinski. 2004. A nanodevice for rectification and pumping ions.Am. J. Phys.72:567–574.
37.) Siwy, Z., and A. Fulinski. 2002. Fabrication of a synthetic nanoporeion pump.Phys. Rev. Lett.89:198103–1-198103–4.
38.) Astumian, R. D. 1997. Thermodynamics and kinetics of a Brownianmotor.Science.276:917–922.
39.) Hu, Q., V. Srindhara, R. P. Joshi, J. F. Kolb, and K. H. Schoenbach.2006. Molecular dynamics analysis of high electric pulse effects onbilayer membranes containing DPPC and DPPS.IEEE Transactions on Plasma Science. In press.
40.) Haest, C. W. M., D. Kamp, and B. Deuticke. 1997. Transbilayerre orientation of phospholipid probes in the human erythrocyte membrane. Lessons from studies on electroporated and resealed cells. Biochim. Biophys. Acta.1325:17–33.
41.) Schoenbach, K. H., S. J. Beebe, and E. S. Buescher. 2001. Intracellular effect of ultrashort electrical pulses. Bio electromagnetics. 22:440–448.

Electrical model of a cell

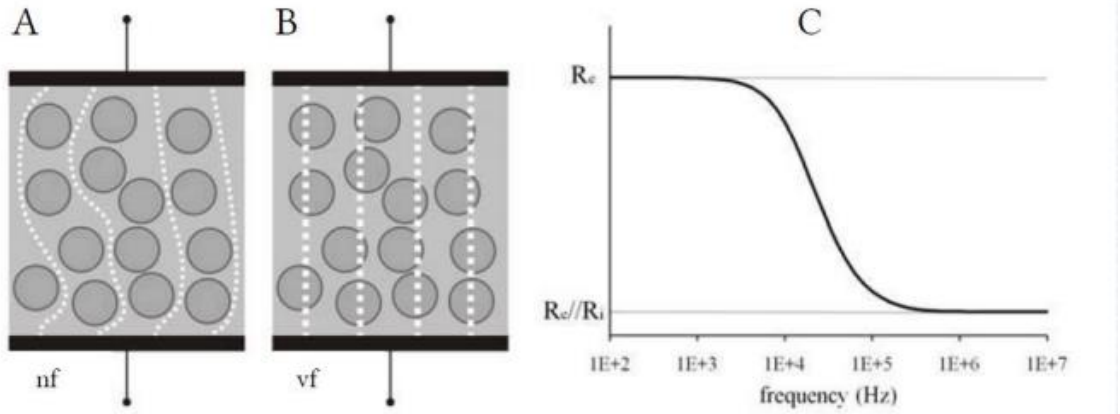


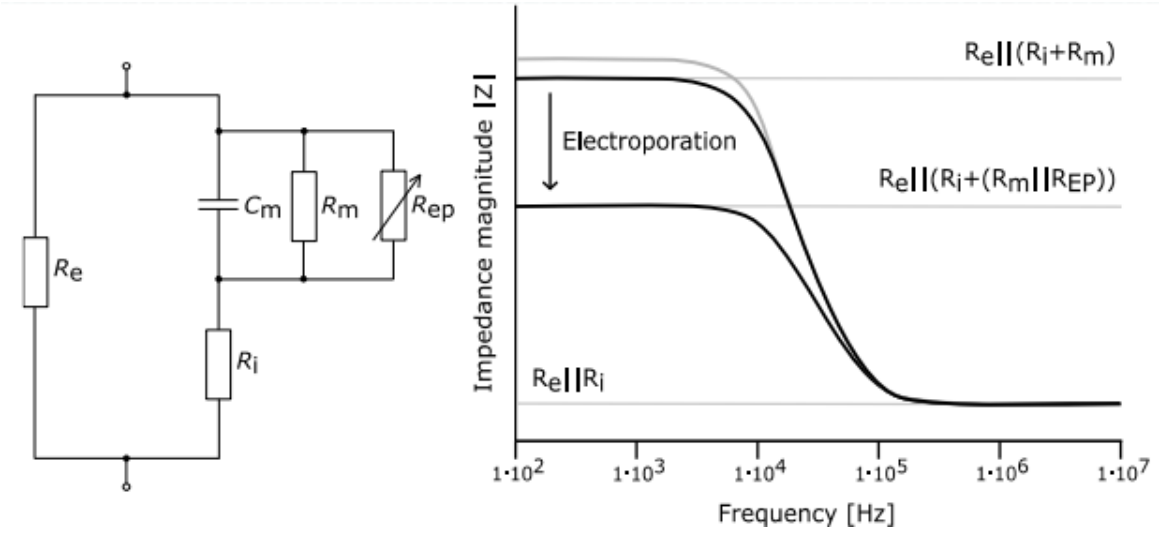
Milieux extracellulaires (plasma sanguin)
ρEM **= 0,66 Ωm à 37°C**

Milieu intracellulaire
ρIM **= 1,66 Ωm.**

Membrane cellulaire
$Cmem$ **= 1 µFcm-2**

Cell model frequency dependence





$$|Z_{f\to\infty}| = R_e||R_i = \frac{R_e \cdot R_i}{R_e + R_i}$$

thank you for your attention

Questions :

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