Electro(magnetic) fields interactions with cells: mechanistic insights and potential new therapeutic applications

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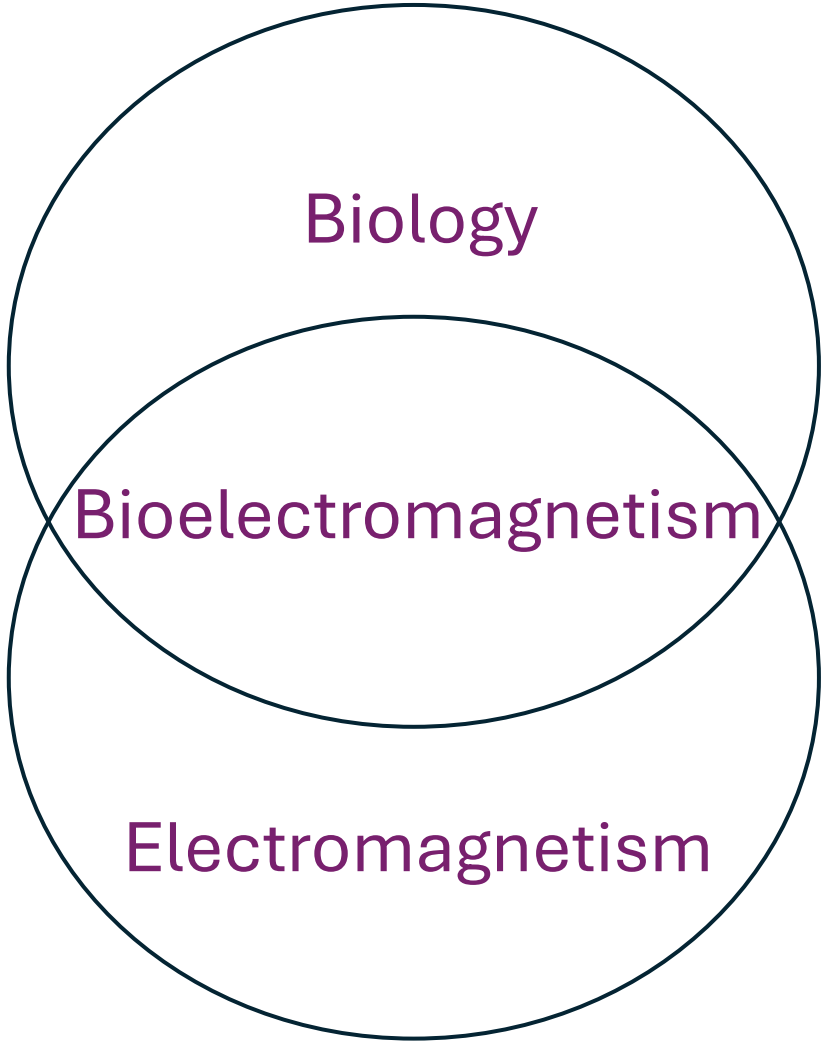
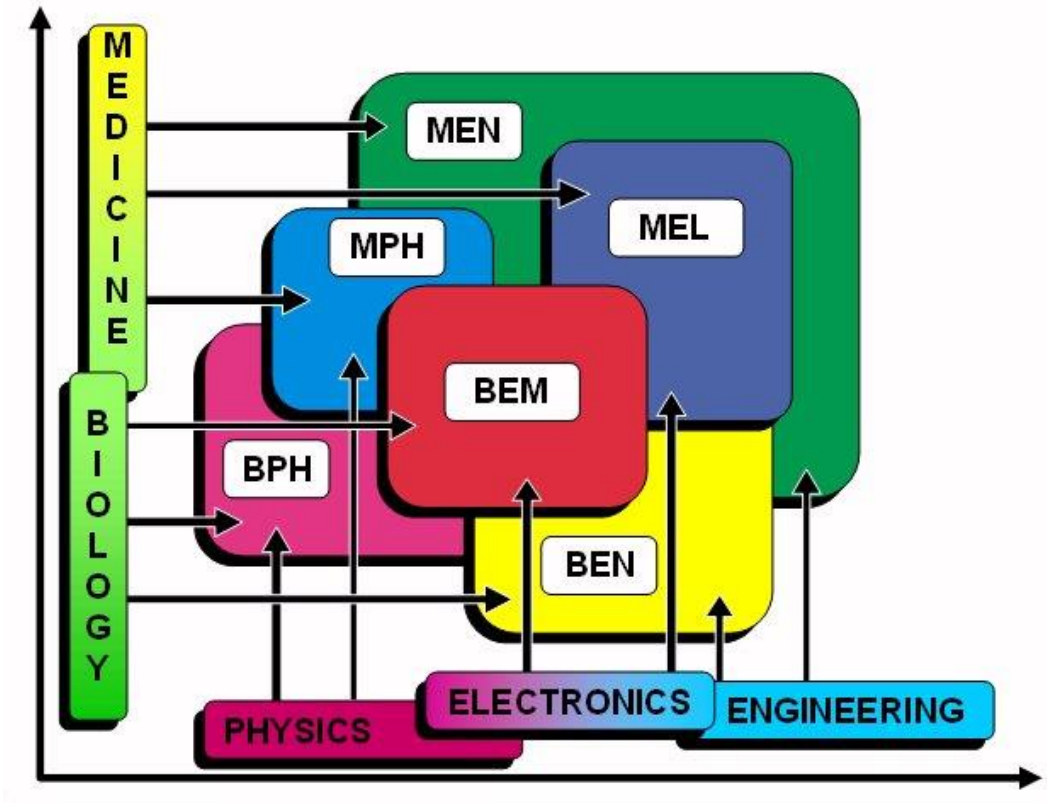
The grayed areas cover figures that are in the process of being published.

The presentation will be updated as soon as the publication process is finalized.

Bioelectromagnetism/Bioelectromagnetics : the study of the interactions between electromagnetic fields and biological systems



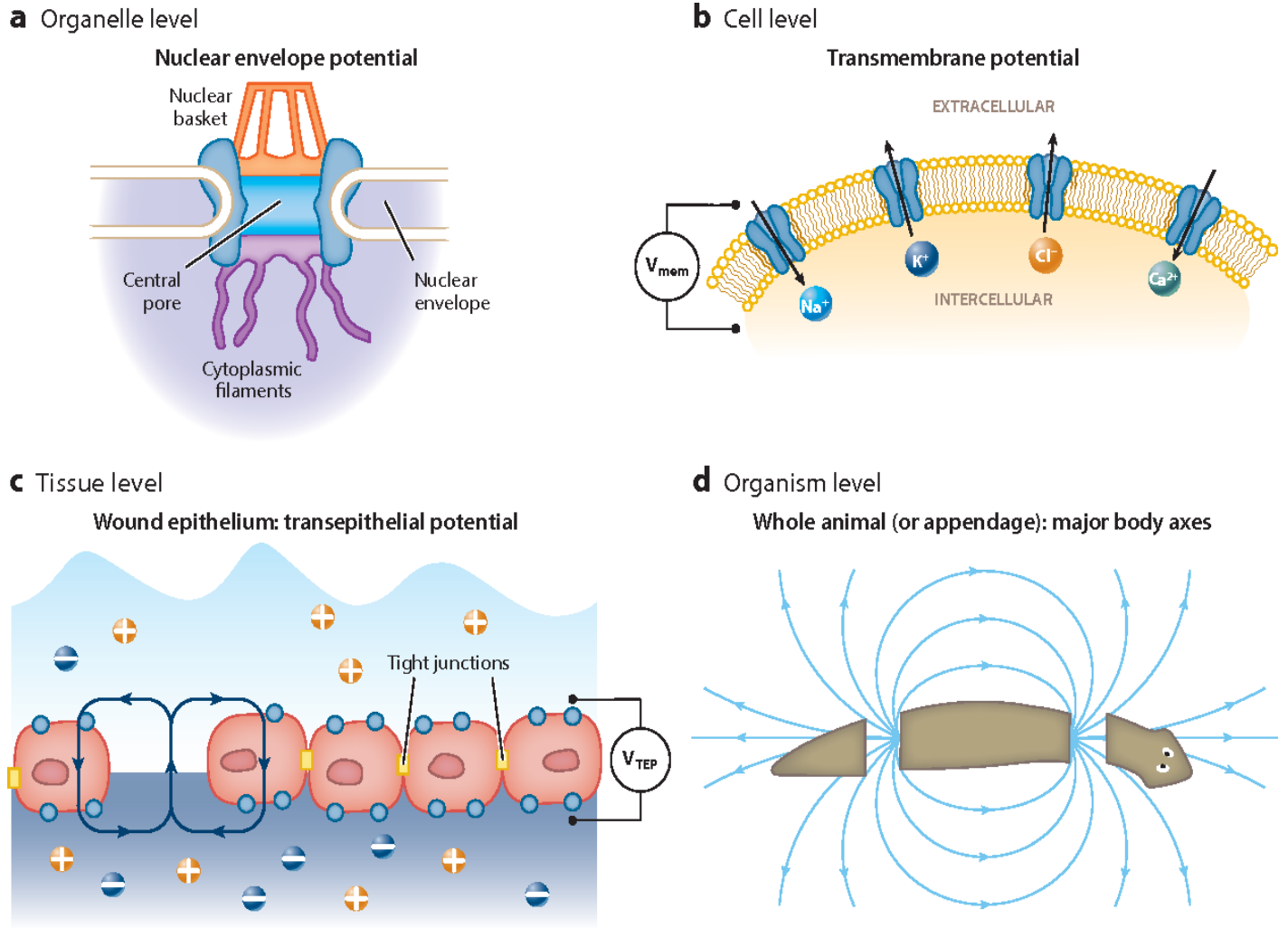


Malmivuo & Plonsey, 1995

BEM: Bioelectromagnetism / BEN: Bioengineering / BPH: Biophysics / MPH : Medical physics / MEL: Medical electronics / MEN : Medical engineering

Bioelectromagnetism: Endogenous physiological electro(magnetic) fields

Observations and lessons learned from physiology



From the **cellular** to the **organism** scale: various **endogenous electric fields** govern numerous **physiological processes**

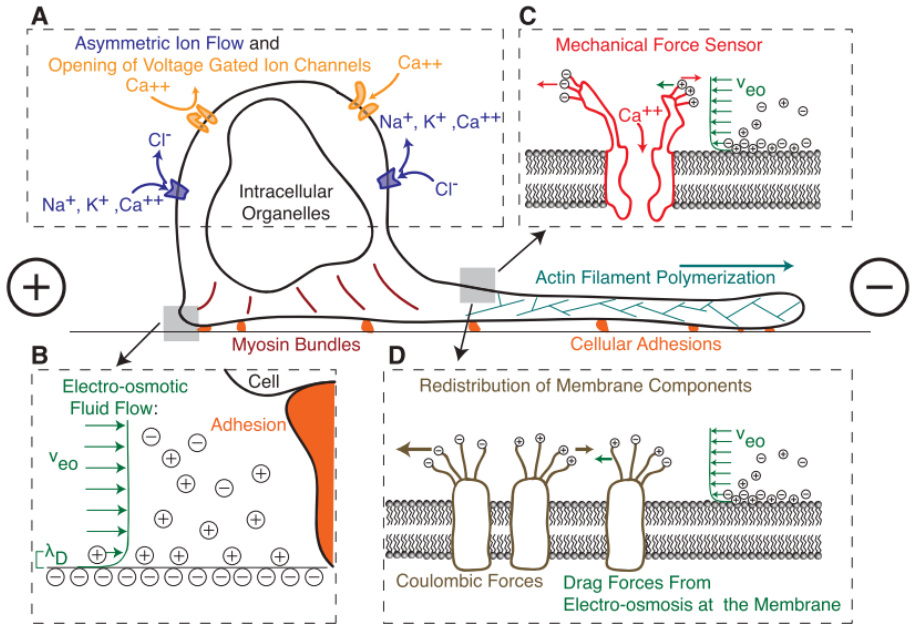
Levin et al. 2007

Bioelectromagnetism: Applying exogenous EMFs to biological systems

*Use of direct current electric fields **(DC-EF)***

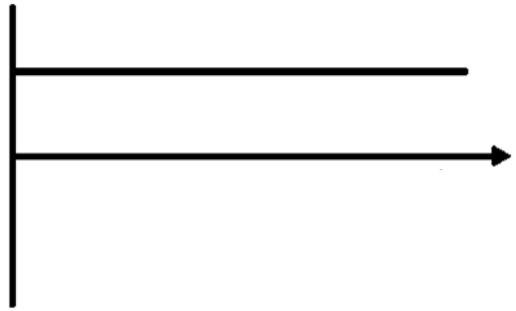
Electrotaxis

Cell motion



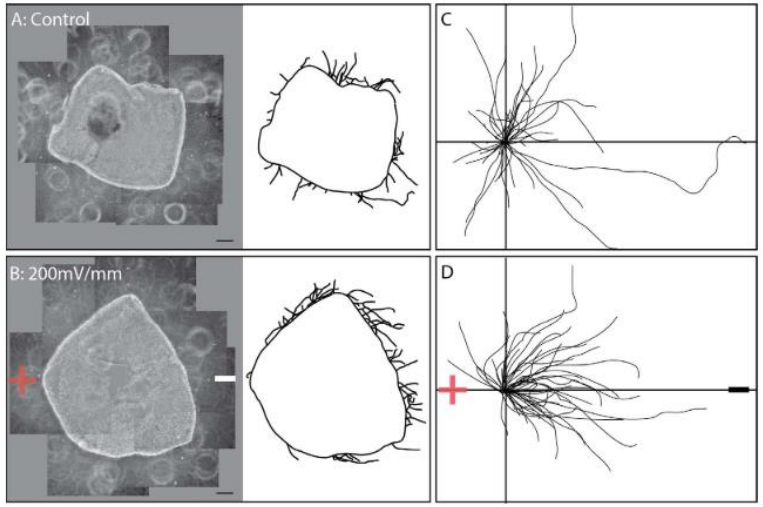
(Allen et al. 2013)

DC-EF



Galvanotropism

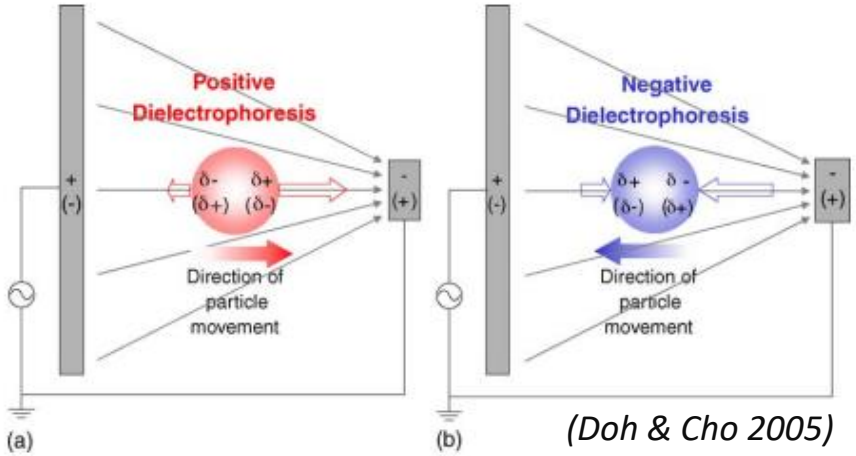
Cell orientation



(Gokoffski et al. 2019)

Dielectrophoresis

Cell electromanipulation

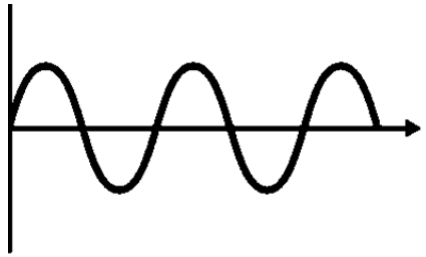


(Doh & Cho 2005)

Bioelectromagnetism: Applying exogenous EMFs to biological systems

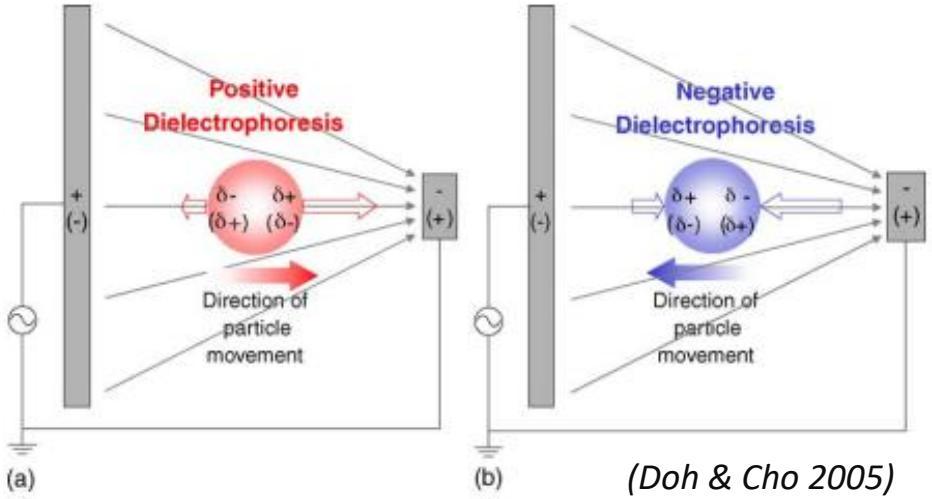
*Use of alternating current electric fields **(AC-EF)***

AC-EF



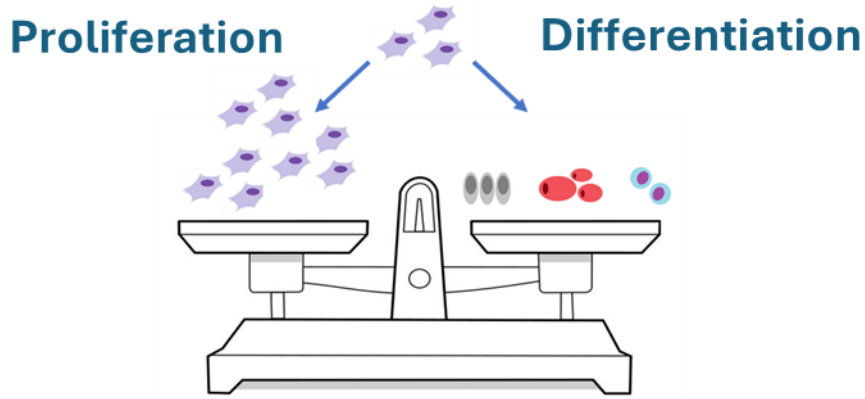
Dielectrophoresis

Electromanipulation / characterization of (di)electric properties

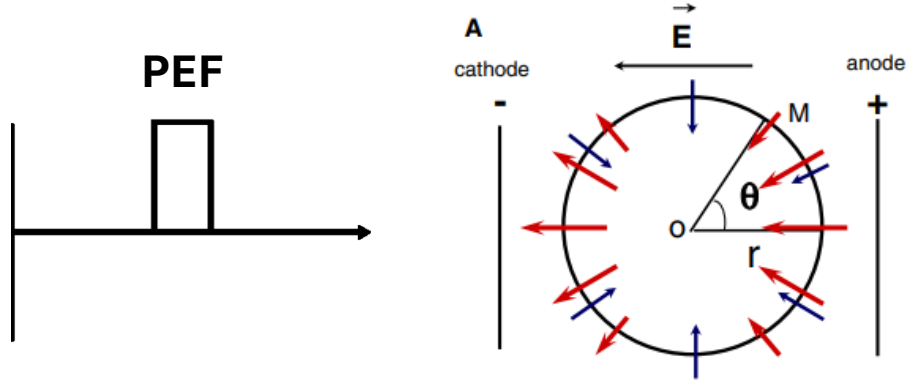


(Doh & Cho 2005)

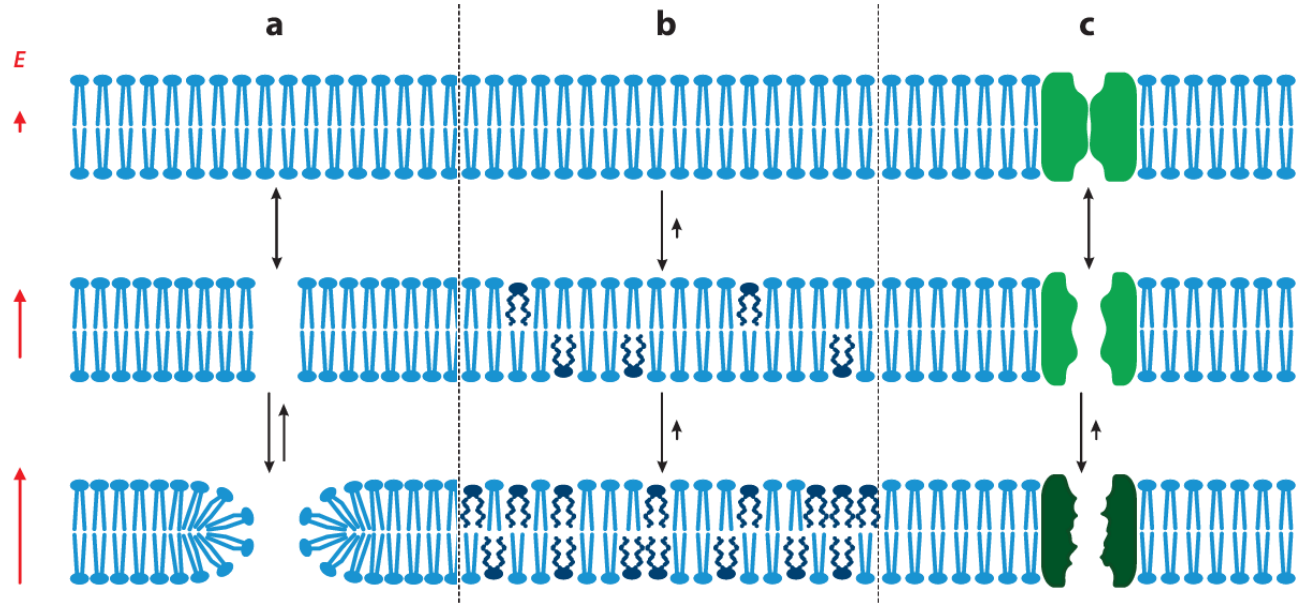
Influence proliferation/differentiation



Use of pulsed electro(magnetic) fields PE(M)Fs
Electroporation/Electropermeabilization



Escoffre et al. 2009



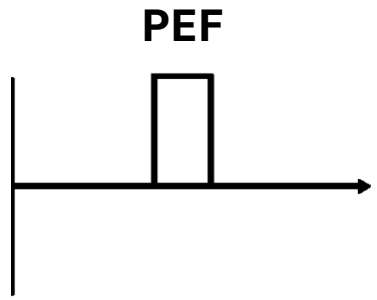
Kotnik *et al.* 2019

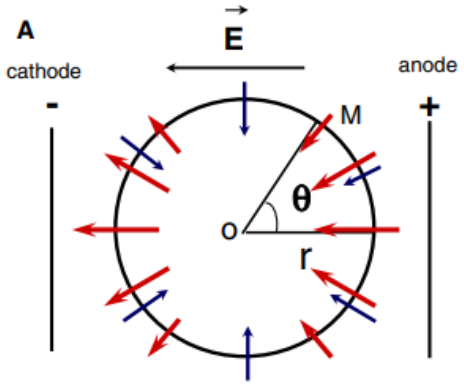
- **Electric Field (E)** induces: **water** and **phospholipid head polarization** + **ions accumulation** at the membrane, modifying **transmembrane potential (ΔΨm)**.
- When **ΔΨm** reaches a **critical threshold**, pores are generated (a).
- **Phospholipids** in the membrane can get **oxidized** (b).
- **Membrane proteins** may be **altered** (c).

Phospholipid

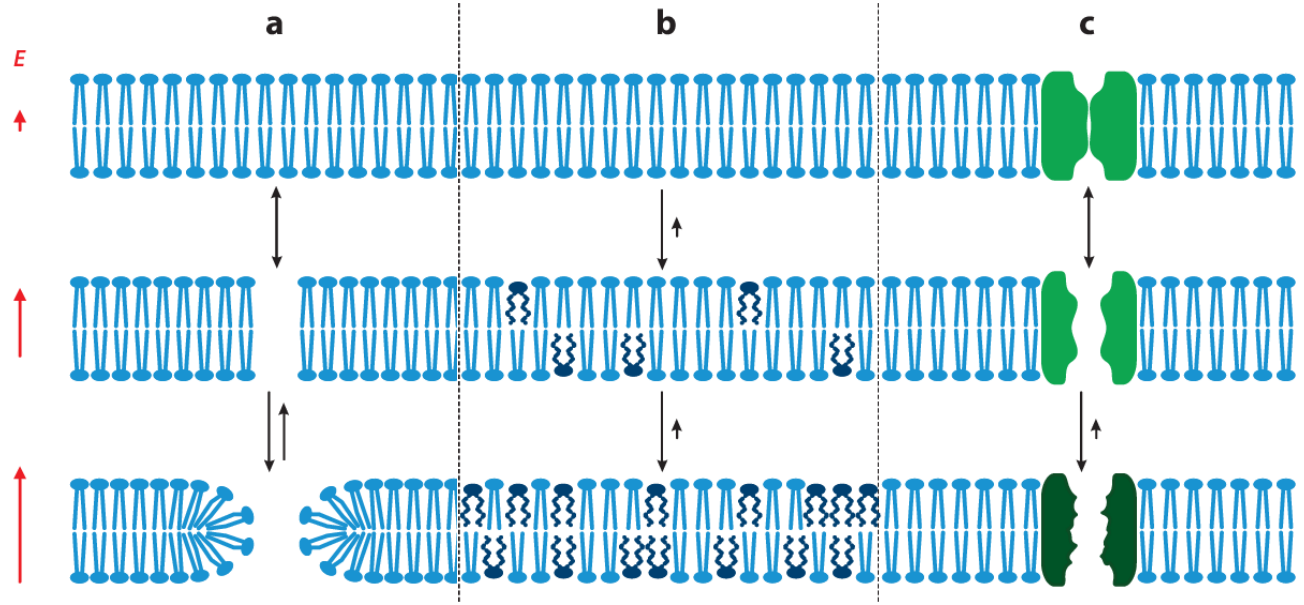
Oxidized phospholipid

Use of pulsed electro(magnetic) fields PE(M)Fs
Electroporation/Electropermeabilization





Escoffre et al. 2009



Kotnik *et al.* 2019

Phospholipid

Oxidized phospholipid

Electroporation/ Electropermeabilization can be **reversible** or **irreversible.**

Therapeutic application of PE(M)Fs : Electrochemotherapy (ECT)

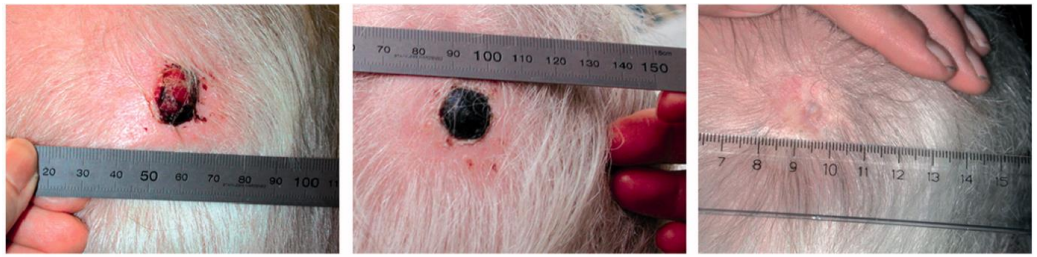
Vectorize

into the cells

Electrochemotherapy = Reversible electroporation + Chemotherapeutic drug (low dose)

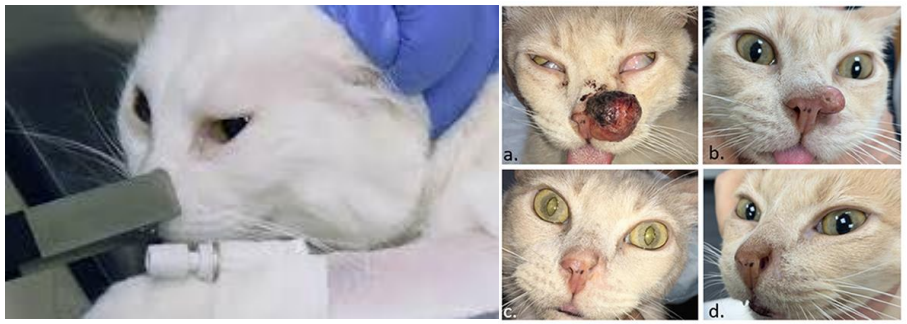
(vectorization mean) (kills cancer cells)

Microsecond PEFs *Bleomycin, Cisplatin*



Before treatment | After 1 month | After 6 months

Gehl et al., 2017



Willows Veterinary Centre, UK *Tellado et al., 2022*

Electrochemotherapy (ECT) – technical considerations

Applying pulsed electric fields requires electrodes in contact with tissues to be exposed

IGEA S.p.A.

Invasive and complex procedures for deep tissue treatment

Surgery, Interventional radiology

What if PEFs could be applied without electrodes in contact ?

Theme 1 : Cell electropermeabilization with subnanosecond pulsed electric fields (SubnsPEFs)

Cell bioelectro(magnetic) property impacted : **transmembrane potential (ΔΨm)**

Desired effect : **Cell membrane permeabilization**

Technological aspects : use of ultrashort PEFs, towards **contactless PEF delivery**

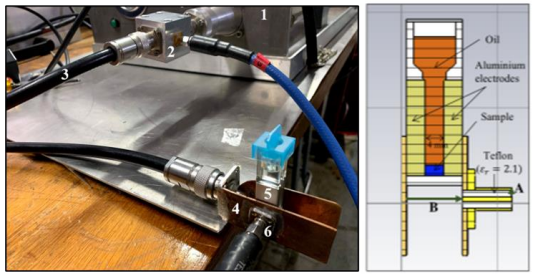
Future applications prospects : performing **contactless/ non-invasive PEF-based therapeutic applications** (e.g., Electrochemotherapy)

Generator delivering SubnsPEFs

Methods

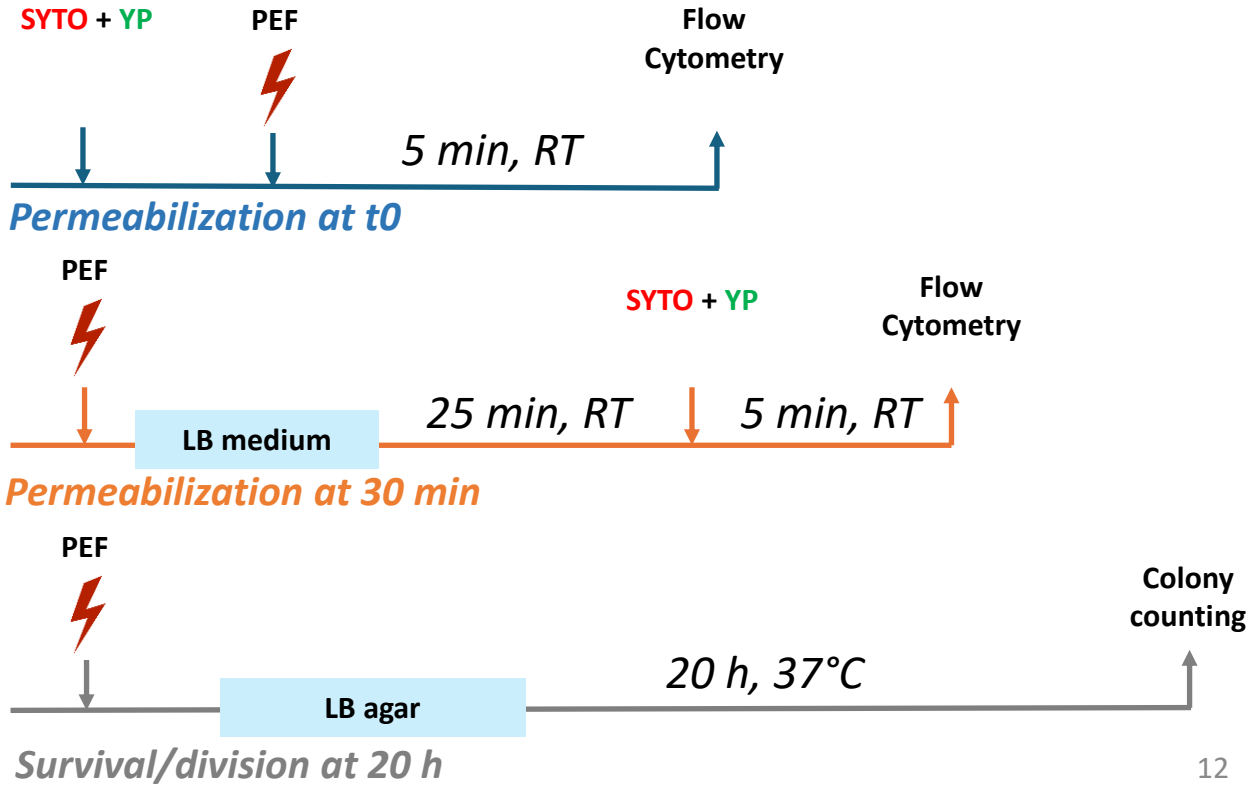
Subnanosecond PEFs applied

Duration : 900 ps FWMH
Rise time : 500 ps



- *E. coli* DH5α in deionized water (low conductivity)
- DNA binding fluorescent stains:
  - SYTO®17 (**SYTO**), can penetrate cells
  - YO-PRO™-1 iodide (**YP**), cannot penetrate cells if the membrane is intact

Experimental set-up



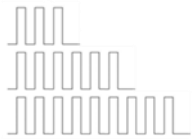
Results of the parametric study

Pulse number

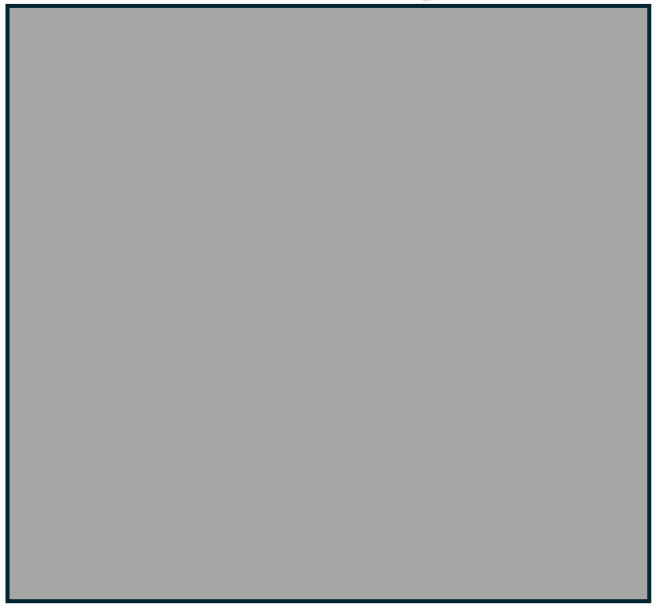
permeabilization at t0
Spearman p (one-tailed) <0,0001

permeabilization at 30 min
Spearman p (one-tailed) <0,01

non-culturability at 20 h
Spearman p (one-tailed) <0,05



- **Cumulative effect**
- **Permeabilization** ↗ ↗ ↗ with number of pulses

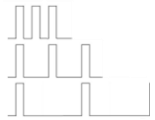


Pulse Repetition Frequency

permeabilization at t0
Spearman, p (one-tailed) <0,05

permeabilization at 30 min
Spearman, p (one-tailed) <0,05

non-culturability at 20 h

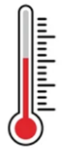


- **permeabilization** ↗ with PRF

Temperature

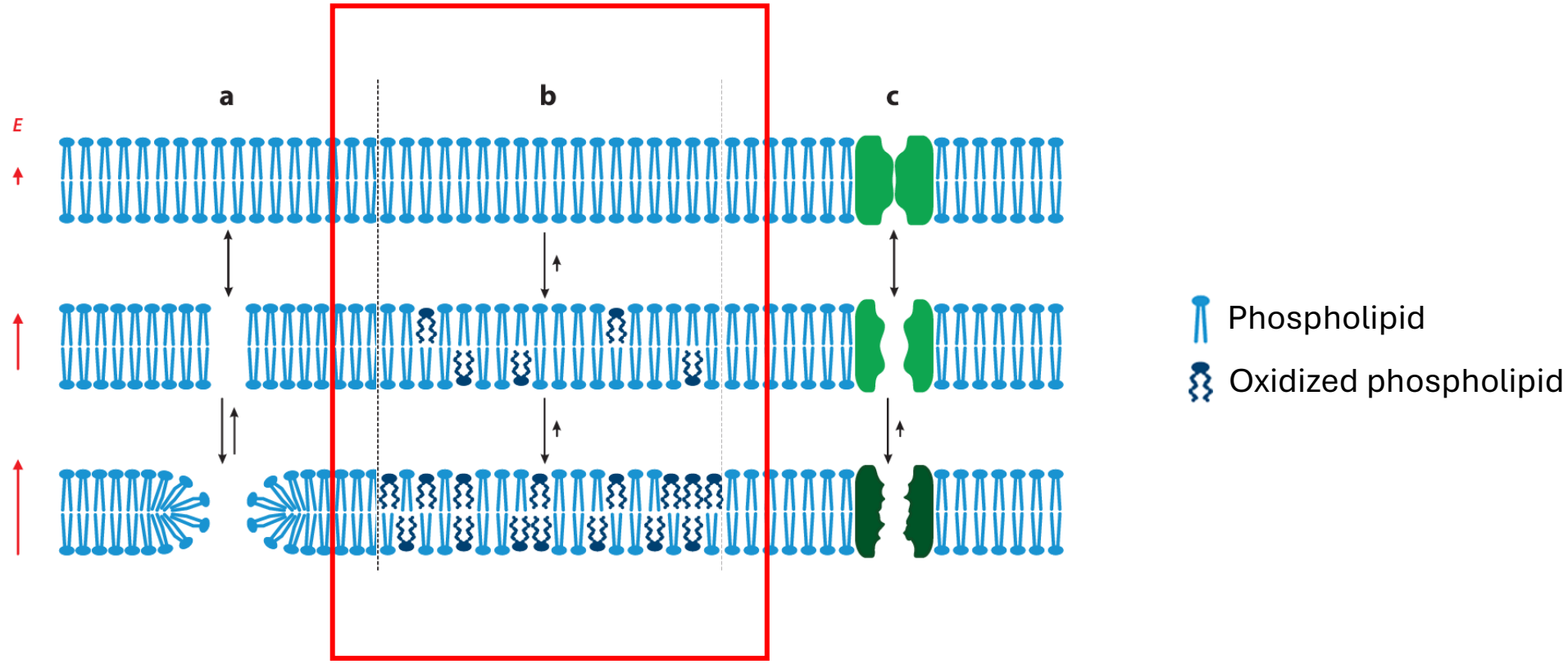
z

one-tailed) < 0,01



- **Permeabilization** ↗ with temperature

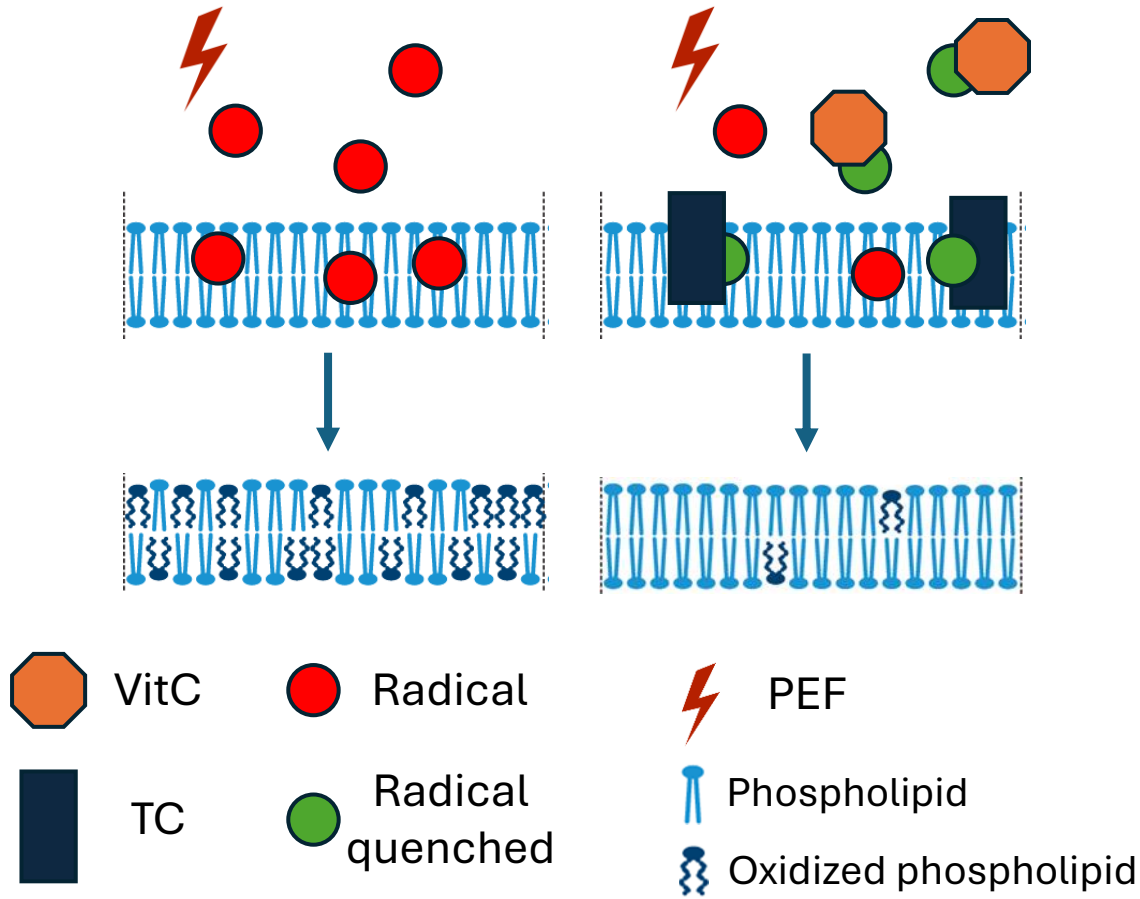
Models of membrane permeabilization



Sub-nanosecond PEFs tested in this study generate a long lasting **permeabilization**

Long-lasting permeabilization can be associated with **oxidative phenomena** at the level of the membrane

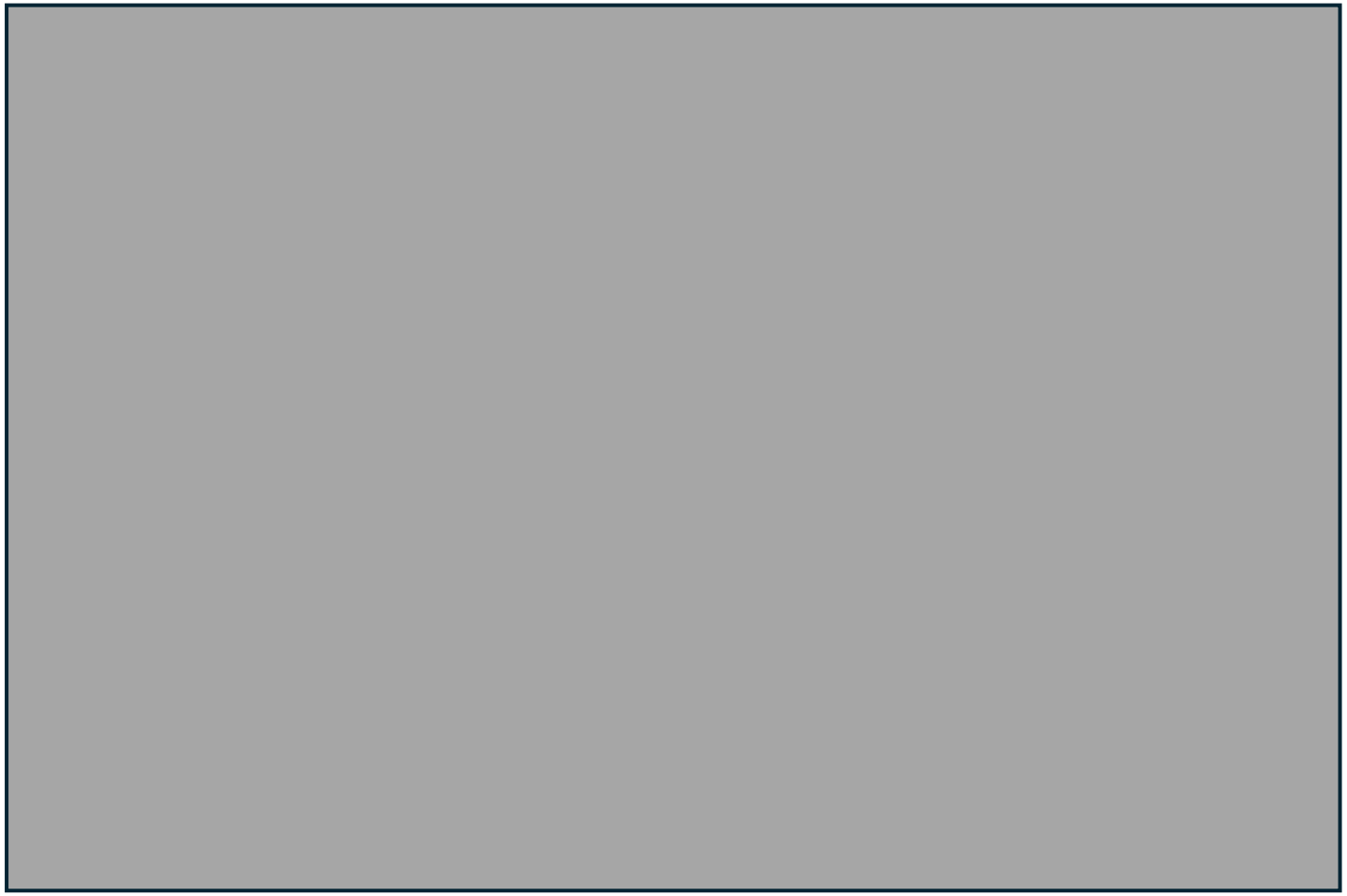
Effect of antioxidants on the permeabilization at low field amplitude



α-Tocopheryl phosphate (TC) + **L-Ascorbic acid (VitC)** in combination ↘ ↘ ↘ **permeabilization**.

The permeabilization at **« low » electric field** and **« low » number of pulses** depends on **oxidative phenomena**.

Peculiarities of sub-nanosecond PEFs



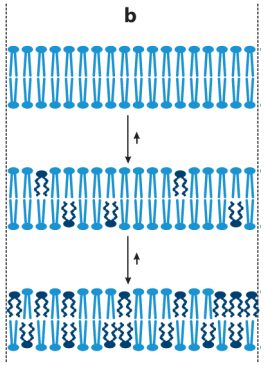
Rise time: **500 ps**

Duration : **900 ps** FWHM

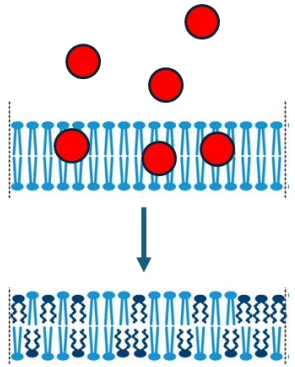
Electric field: **10 à 60 kV/cm**

Effect of the electric field amplitude

Permeabilization with subnsPEFS depends on oxidative phenomena

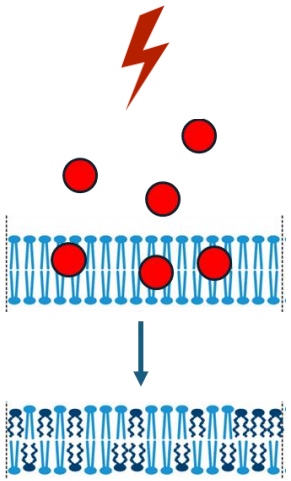


Oxidative phenomena are initiated by radical species



Radical

PEFs are associated with ROS/RNS generation



PEF

Radical

Effect of the electric field amplitude

Conclusions

Subnanosecond PEFs generate **efficient cell permeabilization** at relatively **low electric field amplitude**

Possible to reach these electric field amplitude with **current antenna technologies**

Parametric study:

The **number of pulses**, **pulse repetition frequency** and **temperature** have similar influence as for longer PEFs.

Mechanisms:

Permeabilization generated at « low » electric field amplitude depends on **oxidative phenomena**

Mechanistic hypothesis:

Theme 2 : Using EMFs to characterize and control features of Mesenchymal Stem Cells (MSCs) in proliferation and differentiation

Calcium (Ca^{2+}) oscillations of MSCs in proliferation and differentiation hacking with microsecond PEFs

Cell bioelectro(magnetic) property impacted : **transmembrane potential (ΔΨm)**

Desired effect: **Slight cell membrane permeabilization to Ca^{2+} ions**

Technological aspects: developpment of *in-vitro* exposure system for attached cells to µsPEFs

Future applications prospects: Influence of MSCs cell fate in the context of regenerative therapies

Dielectrophoretic behavior of MSCs in proliferation and differentiation

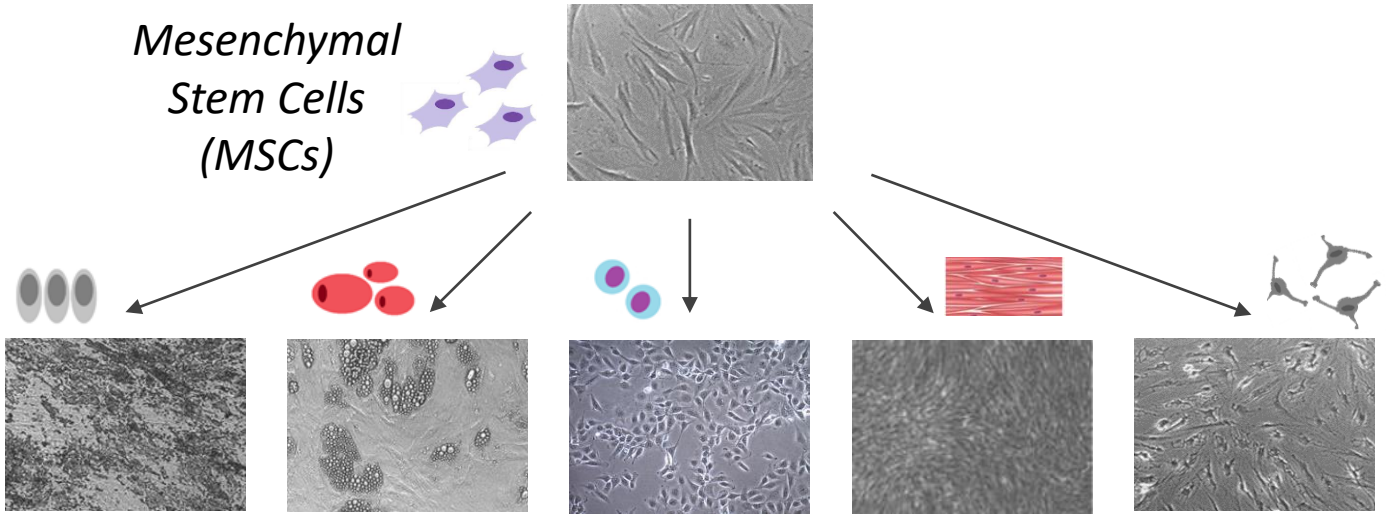
Cell bioelectro(magnetic) property impacted : **cell polarization**

Objectives: Assess (di)electric properties of MSCs

Technological aspects: development of non-damaging separation methologies

Future applications prospects: separation of differentiating MSCs (from early stages) from undifferentiated MSCs, in research or in clinics

Calcium oscillations in mesenchymal stem cells (MSCs)

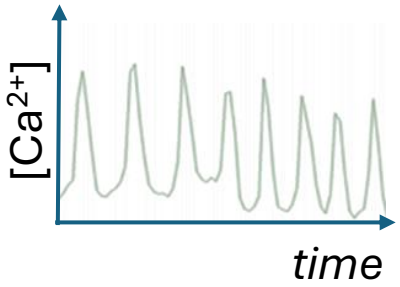


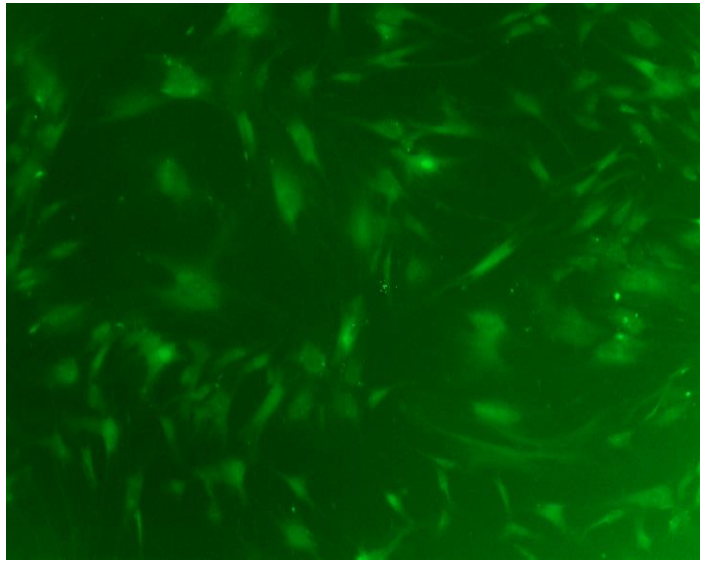
MSCs are **adult multipotent** stem cells

High interest in **regenerative therapies**

MSCs naturally display « spontaneous » Ca^{2+} oscillations

Ca^{2+} oscillation = variation of Ca^{2+} concentration inside the cell with an oscillatory behavior ➔ cell signaling

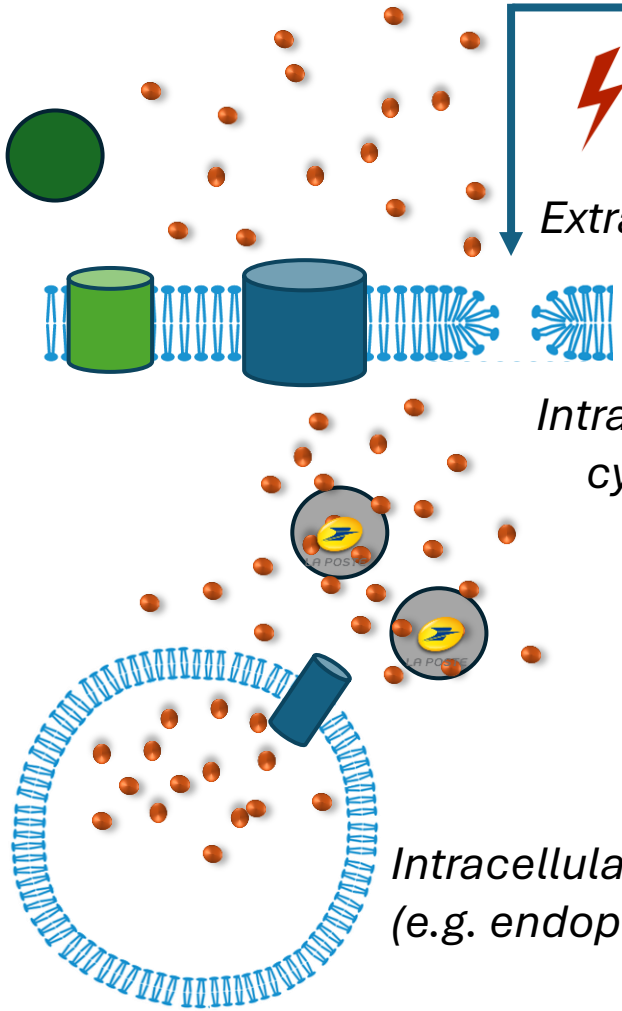


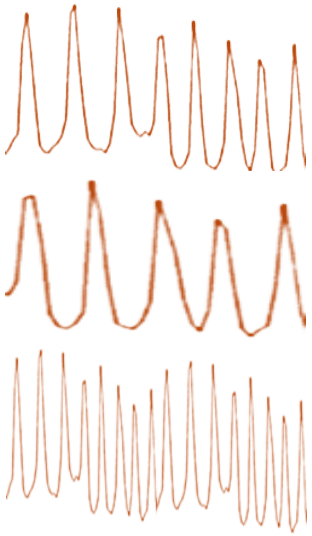


Ca^{2+} oscillations recorded in MSCs with Fluo-4 Ca^{2+} binding fluorescent dye

Controlling Ca^{2+} oscillations to influence cellular processes ?

1 PEF = 1 Ca^{2+} oscillation





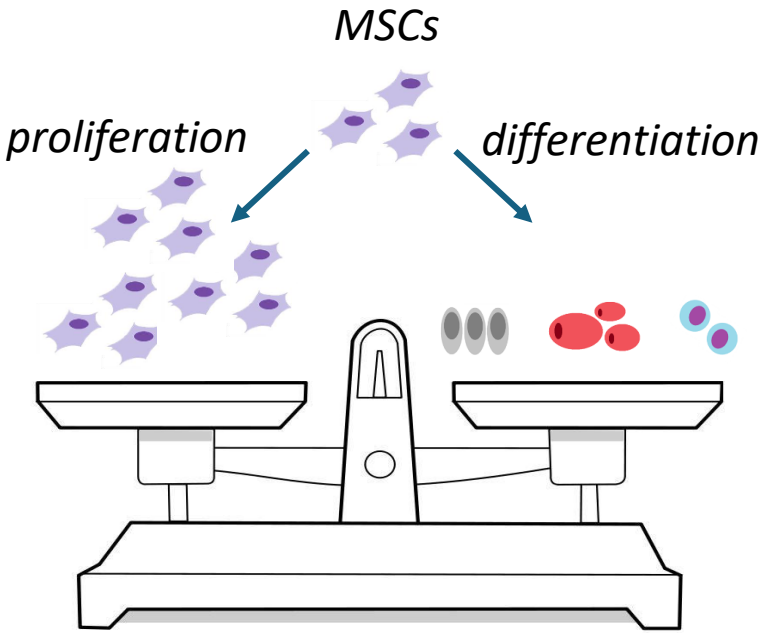
Primary messenger

Calcium ion (Ca^{2+})
Second messenger

Message delivered to the target in the cell

Hacking the system
Manipulation of Ca^{2+} oscillation through reversible cell electroporation

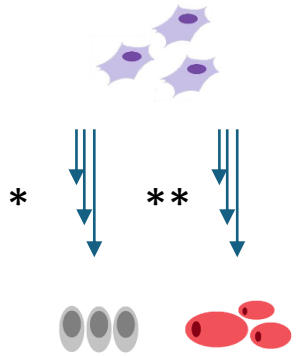
Influence MSCs fate



Calcium ion (Ca^{2+}) | Ca^{2+} channel or pump | Primary messenger | Receptor of primary messenger | Final target of the primary messenger in the cell

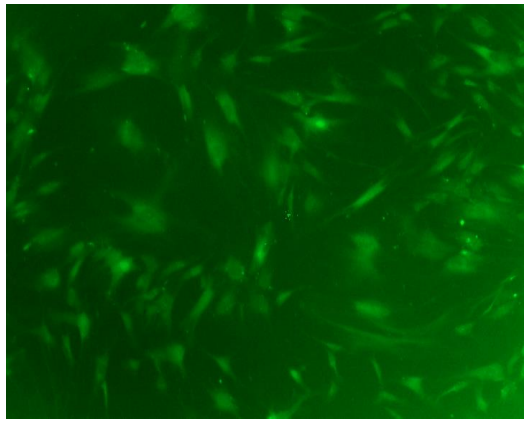
Characterization of Ca^{2+} oscillations in differentiating MSCs

Differentiations

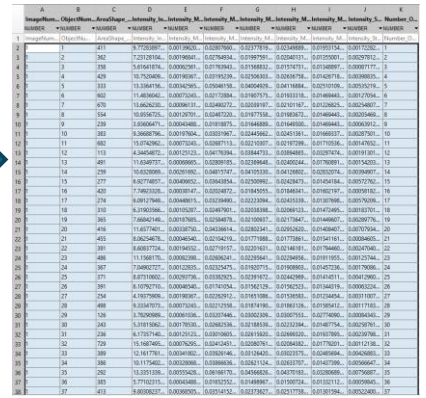


Real-time Microscopy

Fluo-4 AM et Hoechst

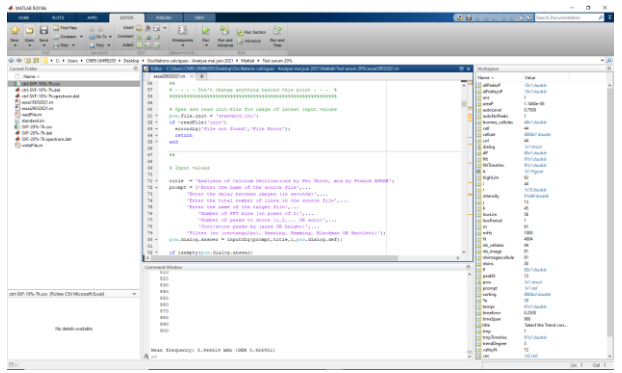


Calcium variations in time



Cell Profiler

Analyzing Calcium oscillations frequencies

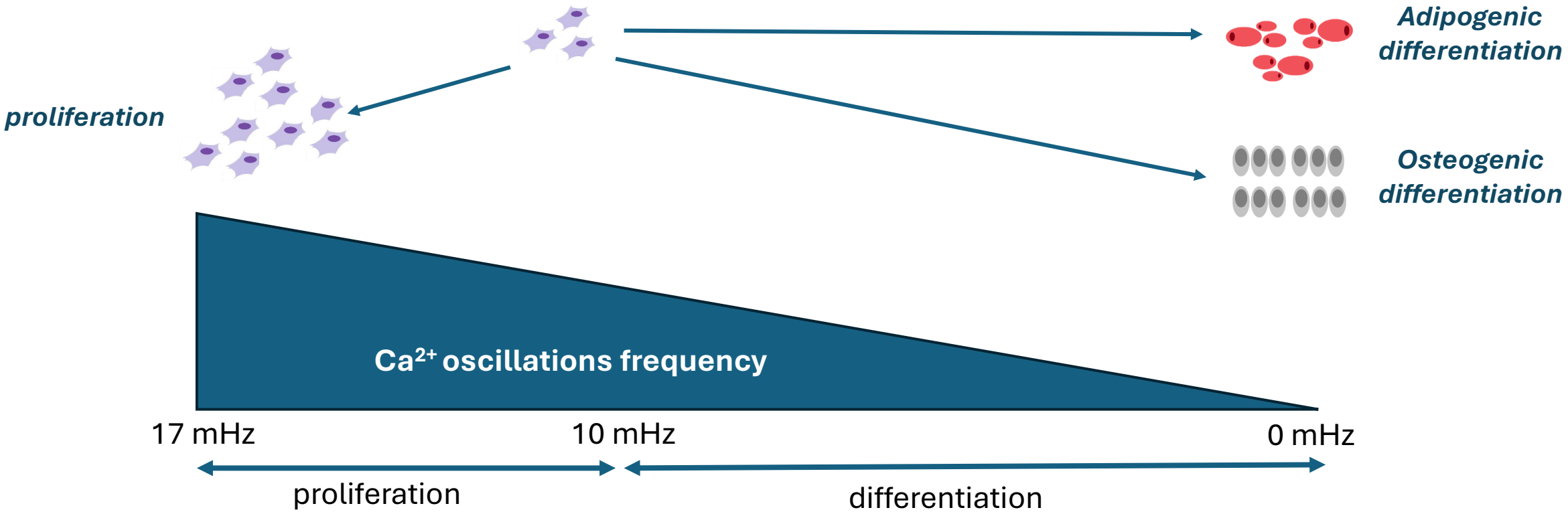


Program inspired from
Spectral Analysis of Calcium oscillations
Uhlen., 2004

* Osteogenic differentiation : complete αMEM + DEX (100 nM) + Ascorbic acid (200 µM) + β-Glycerol phosphate (10 mM)

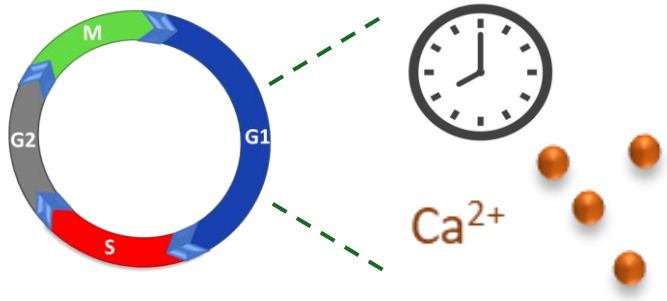
** Adipogenic differentiation : complete DMEM + Insulin (10 µg/mL) + IBMX (500 µM) + DEX (1 µM) + Indomethacin (200 µM)

Evolution of Ca^{2+} oscillations in MSCs in proliferation and differentiation



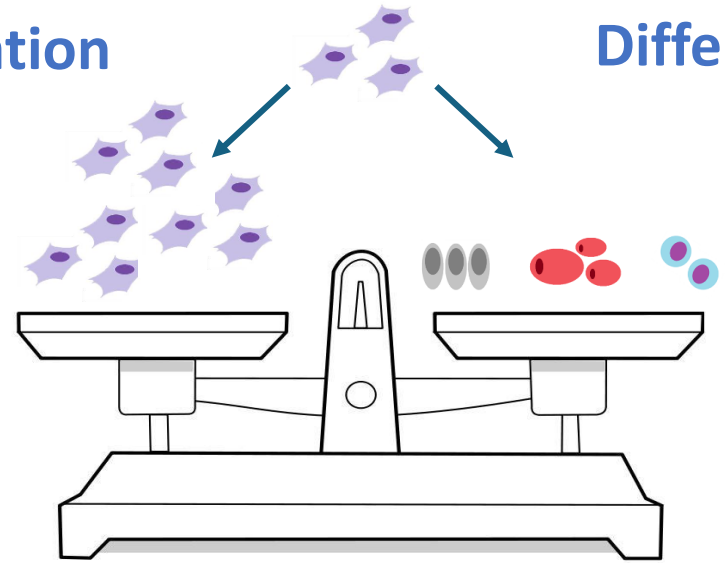
Ca^{2+} oscillations are mostly comprised between 10 and 17 mHz in proliferating MSCs
Ca^{2+} oscillations frequency decreases in differentiation down to full arrest in differentiated state

Electrical stimulation strategies



Proliferation

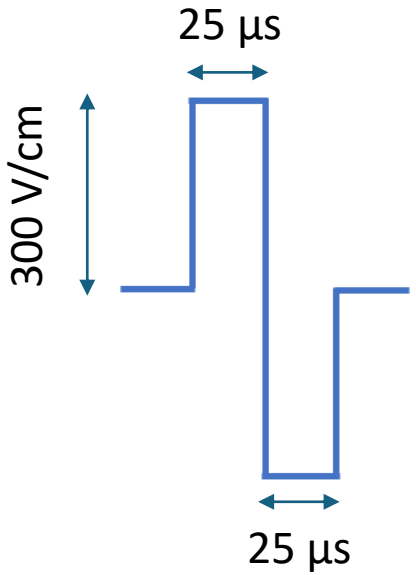
↗ Calcium oscillations frequency



Differentiation

↘ Calcium oscillations frequency
(*in presence of differentiating factors*)

Electrical stimulations and MSC proliferation



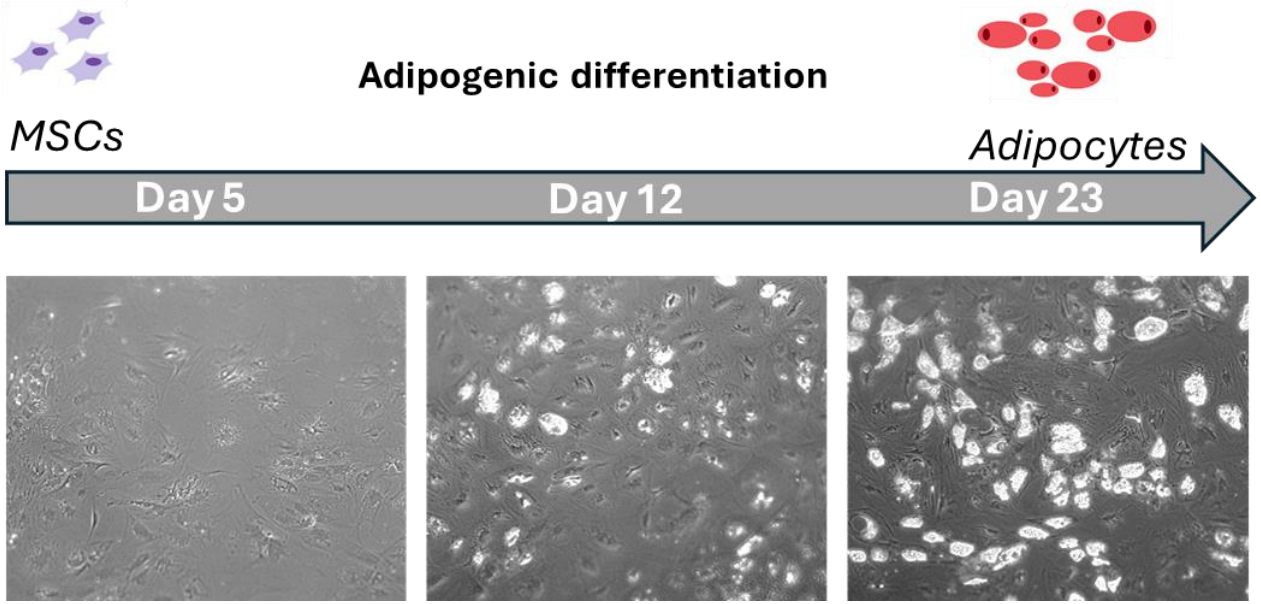
Stimulation with bipolar **25 µs + 25 µs PEFs** at **300 V/cm**

1 pulse/min, for 30 min, 1 cycle/day

Up to 4 consecutive days of treatment

Visible difference in proliferation starting from day 3

Use of EMFs to characterize (di)electric properties of MSCs in differentiation : Dielectrophoresis (DEP)

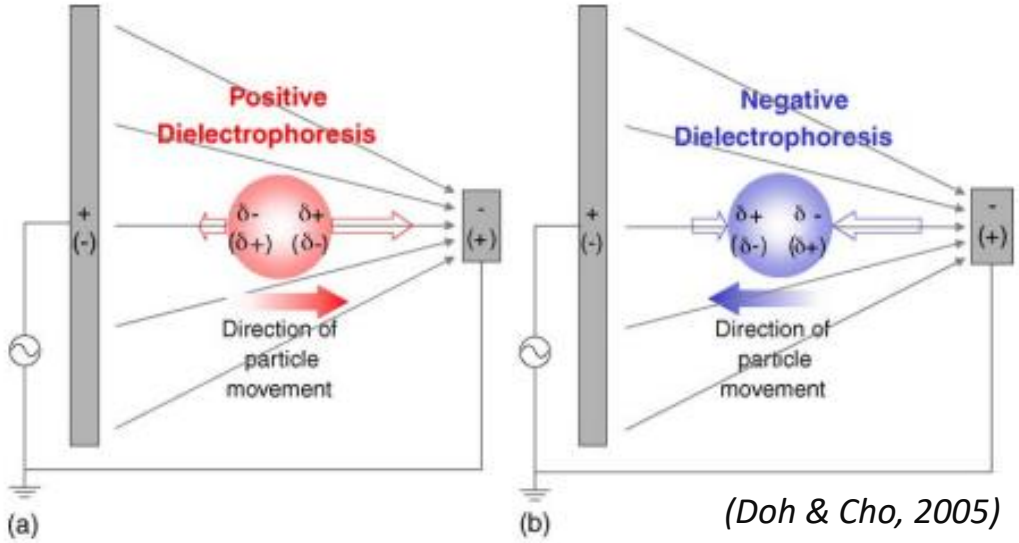


Heterogeneous : Not all cells differentiate

Asynchronous : Cells differentiate at different speed

Challenging to identify MSCs committing to differentiation in the early stages without damaging the cells

Dielectrophoresis (DEP)



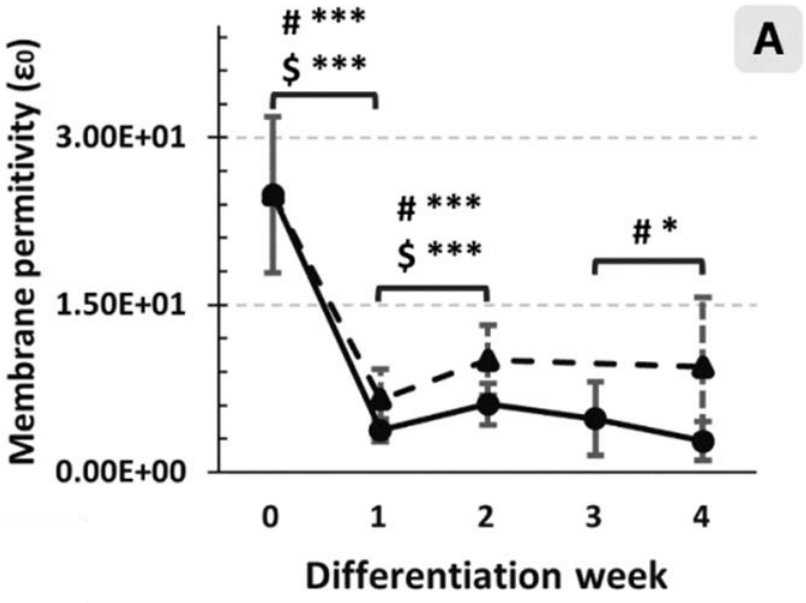
(Doh & Cho, 2005)

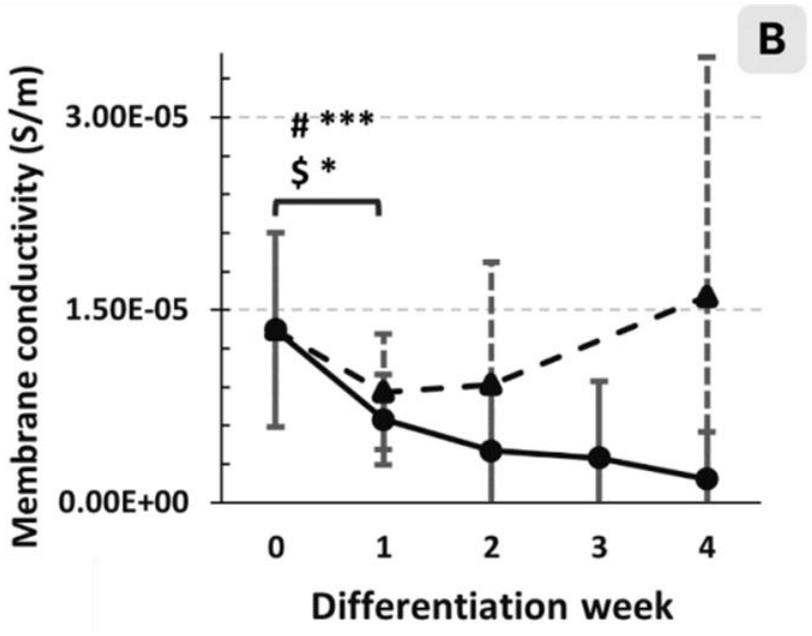
move an **electrically neutral** particule (e.g., a cell) in a **spatial electric field gradient**

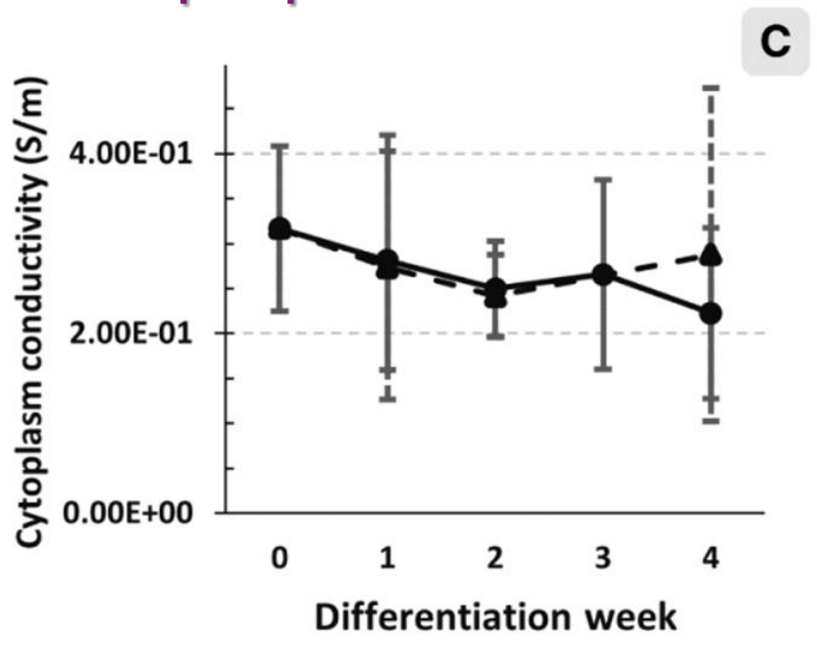
DEP force depends on the **polarization of the particle** with respect to its **surrounding medium**

Polarization depends on the **frequency** of the electric field

DEP on MSCs in differentiation : Cells (di)electric properties





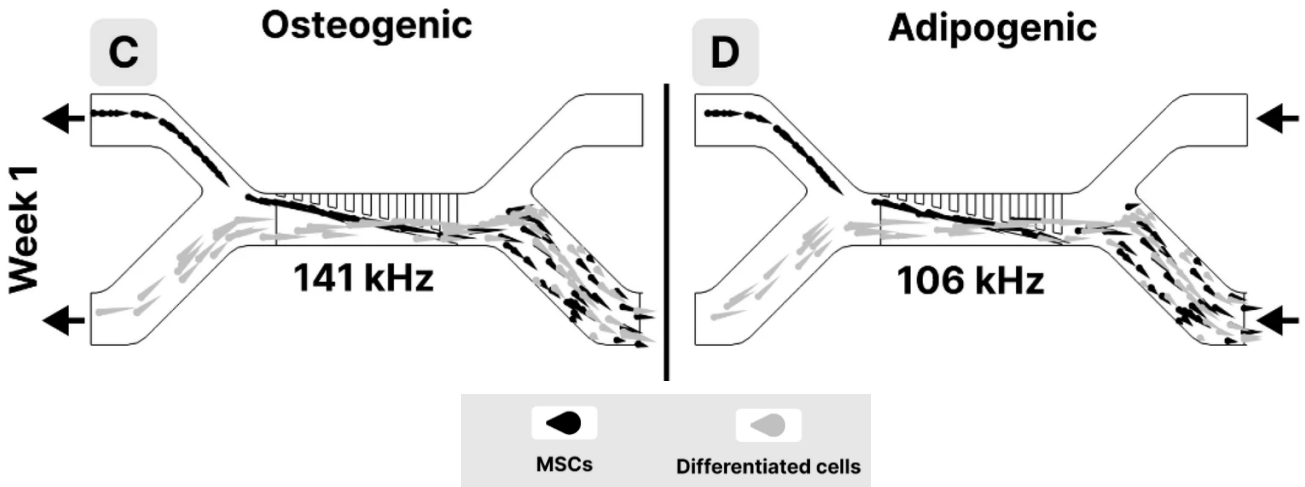


Adipogenic cells — Osteogenic cells

Membrane permitivity ↘↘↘ from week 1

Membrane conductivity ↘ from week 1

Possible to sort undifferentiated MSCs from cells in early differentiation by **DEP** + **microfluidic**



	Cell permeabilization induced by SubnsPEFs	EMFs to characterize and control properties of MSCs	
Fundamental aspects / mechanistics insights	SubnsPEFs induced cell permeabilization - Depends on **oxidative phenomena**	Ca2+ oscillations in MSCs : **Proliferation:** frequencies **between 10 and 17 mHz** **Differentiation: Below 10 mHz**, progressive ↘ down to complete arrest **Microsecond PEFs** can be used to **increase proliferation**	In differentiating MSCs: From **week 1** Membrane **permittivity** ↘ ↘ Membrane **conductivity** ↘ ↘
Applications / medical perspectives	Performing **contactless/ non-invasive** PEF-based **therapeutic applications** (e.g., Electrochemotherapy) *– Ongoing assessment of cell permeabilization with contactless SubnsPEFs*	Influence **MSCs fate** in the context of **regenerative therapies** with **PEFs** *– Ongoing research in RISEUP project aiming at regenerating spinal cord in spinal cord injury*	Separation of MSCs in differentiation (from early stages) and undifferentiated MSCs (**enrichment/ purification**) for use in **research** or in **clinics**

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Thank you for your attention