Novel and versatile instrumentation for electro-manipulation of cancer stem cells

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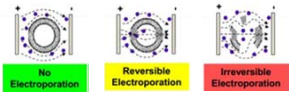
Introduction to the Project

- Part of European Union's Horizon 2020 research and innovation program: **Semiconductor based Ultrawideband Micromanipulation of CAncer STem Cells** or **SUMCASTEC**
- SUMCASTEC explores a new approach for real time isolation and neutralization of Cancer Stem Cells (CSCs). **http://www.sumcastec.eu**
- CSC are associated with Glioblastoma Multiforme (GBM) and Medulloblastoma (MB) relapse [1].
- A project deliverable: to develop an off-chip pulsed Electric-Field (EF) generator for cell electro-manipulation.

Cell electropermeabilization:



- Alternative physical technique for non-thermal treatments
- Use precisely controlled **high amplitude pulsed electric fields** of **short duration (ns, µs)** to alter the cell's transmembrane potential
- Results in permeabilizing the cell's plasma membrane and disturbing intercellular homeostasis
- The resultant permeabilization of cell plasma membrane can be reversible or irreversible.

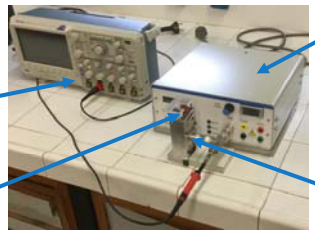
One of the Project goal:

- To deliver a generator capable of pulse amplitude in excess of 1 kV, with pulse widths in the hundreds ns regime for cell electro-manipulation.
- Minimisation of overshoot and ringing (Flat-Top pulses)
- Investigate various pulse parameters associated with the SUMCASTEC Pulse Generator (SPG) on CSC. (pulse duration, repetition frequency, number of pulses)
- To developed a non-thermal treatment
- Investigate SPG effects on CSC suspended in a 50 Ω buffer and other conductive solutions.

Instrumentation

Dynamic monitoring of pulse during EF delivery (Oscilloscope +High voltage probe)

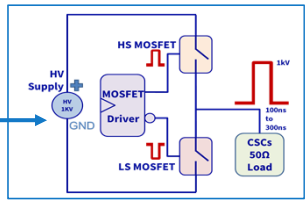
SUMCASTEC Pulse Generator (SPG)



Bio-rads 0.1 cm gap Cuvette containing CSC and a 50Ω artificial buffer

ENEA Cuvette Housing Unit

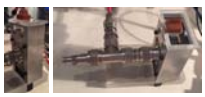
- Push-pull switching of High Voltage (1700 V), fast switching (70.5ns) MOSFETs
- Driven by opto-isolators with comparable switching times – high enough current to charge up gate-source and gate drain capacitances
- High Side MOSFET determine pulse width. Low-side complimentary of High-side MOSFET : to ensure symmetrical fall time.



SUMCASTEC Pulse Generator (SPG)

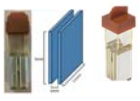
- Pulse repetition Frequency: 1 - 50 Hz
- Pulse Width Generated: 80 ns - 1 µs
- Number of pulses generated: 1 - ∞ infinite (continuous wave)
- Pulse amplitudes: 280 V – 1200 V
- N-connector and banana sockets output.

ENEA Cuvette Housing Unit



- Exposes CSC in cuvette compatible with CSCs electro-permeabilization (EP) connected with standard N-connectors
- Allow real time monitoring of high voltage (HV) electric pulses.

ENEA 50Ω artificial buffer



- Buffer = PBS + H2O + Sucrose [2]
- Proportions for 100 mL of 0.3 S/m (50Ω) buffer:
  - 20 mL of PBS (phosphate saline buffer)
  - 80 mL of H2O (distilled water)
  - 8.2 g of sucrose (to balance out osmotic pressure with the cells)

Conclusions and Acknowledgements

- Real-time pulse visualization
- Positive results obtained in matching strategy
- Non-thermal permeabilization of CSCs
- Successful permeabilization of the CSCs to YOPRO-1 at 200 and 300 ns
- System ready for SUMCASTEC experiments!!!

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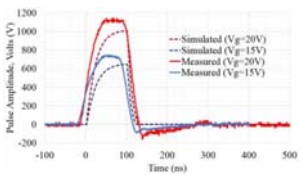
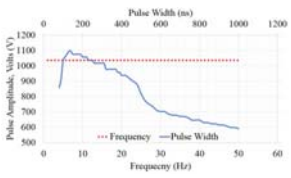
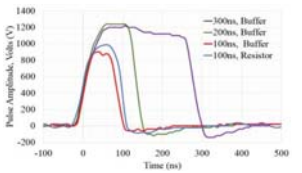
SPG characterization



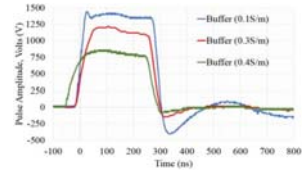
- Flat pulses free from ringing and overshoot
- Increase of gate voltage from 15 V to 20 V results in increased pulse amplitude.
- Developed generator performance exceeds the LTSpice simulation.



- Pulse amplitude is unaffected throughout its operating repetition frequencies (1-50 Hz)
- Optimized in the pulse width range of 100 ns to 300 ns, for pulse amplitudes in excess of 1kV



- Artificial 50Ω is comparable to waveform measured with a 50 Ω resistor.
- 100 ns, 200ns and 300 ns pulse waveforms measured across the EP cuvette containing CSCs suspended in 50Ω, 0.3 S/m buffer solution

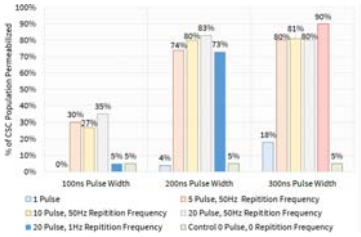


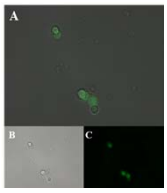
- 300ns pulses with various buffer solution at load
- Shape of the pulse is non-affected
- Demonstrating broadband matching performances

Assessment of CSCs permeabilization

D283 and D341 cell lines from ATCC were cultured in complete Minumum Essential Medium (MEM) supplemented with 10% fetal bovine serum and 1% penicillin streptomycin. The cells were routinely passed each four days. To characterize the level of CSCs in our cell lines, multiple stemness markers were evaluated by wester blot analysis.

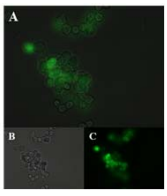
Electropermeabilization Occurrence in D283





Permeabilized cells: 200ns, ~1.1 MV/m, 20 pulse, 1 Hz = 90%

Reversible EP



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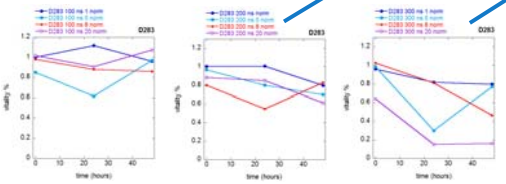
Irreversible EP



D283 viability (trypan blue) assessed after EP (t=0), and at t=24, t=48 and t=72 h after the exposure. Different pulse durations and pulse number were tested at a repetition rate of 1 Hz for pulse amplitude of nearly 1.1 MV/m.

A non-thermal treatment

Pulse Width, P_w (ns)	Amplitude V (kV)	Load, Z (Ω)	Power (kW)	Repetition Frequency, f (Hz)	Energy, E (mJ)	Temperature Change, ΔT (μ^0C)
100	1.0	50	20.0	1	2.00	4.8
100	1.0	50	20.0	50	100.00	239.2
200	1.2	50	28.8	1	5.76	13.8
200	1.2	50	28.8	50	288.00	689.0
300	1.2	50	28.8	1	8.64	20.7
300	1.2	50	28.8	50	432.00	1033.0

$$\Delta T = \frac{\left(\frac{V^2}{Z}\right) \cdot P_w \cdot D}{C \cdot L}$$

Non-thermal effect. of $1.0 \times 10^{-3}\ ^0C$ ($100\mu^0C$)

-D is duty cycle (*ratio*) -E is energy (*J*) -C is heat coefficient, 4.18 J/g/^0C, as buffer mainly consists of water

-L (*indicating volume*) is millilitres (the cuvette can hold 0.1 *mL* of solution)

References

[1] "Tumor Types - National Brain Tumor Society", *National Brain Tumor Society*, 2018. [Online]. Available: http://braintumor.org/brain-tumor-information/understanding-brain-tumors/tumor-types/. [Accessed: 26- Jan- 2018].

[2] M. Caterina, A. Casciati, M. Tanori, B. Tanno and M. Mancuso, "SUMCASTEC_180123_NA_protocolWP3_protocol_.pdf_Rome_C. Merla_Partners and public_NA", *Zenodo*, 2018. [Online]. Available: https://zenodo.org/record/1157784#.Wm9N3a5I-po. [Accessed: 26- Jan- 2018].

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