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

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This paper presents novel instrumentation combined with an artificial 50 Ω buffer for possible fast neutralization of cancer stem cells. The results from an initial bench study investigates the performances of a developed electroporation generator capable of delivering non-thermal treatments in combination with an original cuvette housing unit containing suitable solutions of cancer stem cells. Initial investigation for electropermeabilization threshold of cancer stem cells was conducted, indicating that the developed devices and protocols have a strong potential in achieving electro-manipulation of this biological target.

Introduction

As part of the European Union's Horizon 2020 research and innovation program, the SUMCASTEC project explores a new approach for real time isolation and neutralization of Cancer Stem Cells (CSCs), as Glioblastoma Multiforme (GMB) and Medulloblastoma (MB) via a novel micro-optofluidic lab-on-chip platform [1]. An element of the deliverables is to develop off-chip electric pulse generators in the rage of nanosecond and sub-microsecond, and off-chip applicators to target CSCs. One of the goals is also attempting to understand the effect of applied fields for CSCs neutralization [1].

GBM and MB are primary brain tumors, occurring in adults and children respectively. Brain cancer results in more death per person than any other cancer with very poor outcomes; indeed, the 5-year survival rate is around 4% [2]. The recurrence and relapse of these tumors seem related to the presence of quiescent cancer stem cells that evade common therapeutic approaches (i.e. chemotherapy and radiotherapy).

Cell electromanipulation using very short (down to few hundreds of picosecond) and intense electric pulses (up to tens of MV/m) named also electropulsation is a relatively novel physical technique. Pulsed electric fields alter a cell's transmembrane potential. This results in permeabilizing the cell's plasma membrane and disturbing intercellular homeostasis. The resultant permeabilization of cell plasma membrane can be reversible or irreversible leading in this case to cell death [3]-[7]. Irreversible electroporation is used

for cancer ablation, and has been successfully performed intraoperatively, laparoscopically and percutaneously [3]-[7].

The electric field based neutralization of CSCs could be mediated by cell electropermeabilization, but CSCs differentiation/modification in the absence of electropermeabilization seems another interesting possibility. Usually, the electric pulse duration and repetition frequency are low enough to ensure that the energy delivery into the biological system is non-thermal.

Non-thermal approach seems a preferred method for neutralizing CSCs. This method allows targeted neutralization without heating the CSCs above body temperature. The non-thermal approach predominantly spares normal tissues allowing tissue regrowth and preventing unwanted damage.

In this abstract we present the combination of generators and applicators and their matching to performed off-chip experiments on CSCs.

Instrumentation

One of SUMCSTEC's technical milestones was to build off-chip generators in the sub-microsecond and nanosecond electric pulse regimes. One of this system has to be capable of delivery pulse amplitude in excess of 1 kV, with minimal pulse widths of 100 ns. Its modular design is based on a push-pull switching of high voltage, fast switching MOSFETs that are directly driven by opto-isolators with suitable switching times.

The ability of the modular design to generate the required pulses is dependent upon the switching times and maximum drain-source voltage of the MOSFET. The MOSFET gate driver must also provide enough current to charge the gate-source and gate-drain capacitance. The performances of the developed generator named "SUMCASTEC generator 1 SPG1", presented in Fig. 1, are compared with a built LTSpice model. We demonstrate that our prototype exceeds the LTSpice simulation results across a purely 50 Ω resistor load (data not shown).

In addition to producing 1 kV, 100 ns pulses, SPG1 control and programing allow for a wide range of pulse profiles to be generated spanning from 100 ns up to 1 μs and amplitudes in excess of 1 kV. We also verified that pulse amplitude is unaffected throughout its operating repetition frequencies between 1 to 50 Hz.

Exposing the CSCs (suitably characterized D283 cell line) to high voltage, 100 ns-300 ns pulses were delivered via a cuvette housing unit designed by ENEA. This unit allows commercially available electroporation cuvettes with the CSCs suspended in a specific 50 Ω buffer (phosphate buffer saline and distilled water in a suitable dilution [9]), to be exposed to pulsed electric fields. The housing unit allows an easy connection with the generator (standard N connector) with the possibility of pulse monitoring on an oscilloscope during pulse delivery. An optimized transition between the coaxial connector

and the planar electrode of the EP cuvette was also realized and simulated. Fig. 1 shows the connection of the exposure unit with the SPG1 and its main features.

An artificial 50 Ω buffer, of 0.3 S/m conductivity, to suspend the CSCs was initially used to optimize impedance matching between the SPG1 and the CSCs load. The buffer consists of 20 mL of phosphate saline buffer, 80 mL of distilled water and 8.2 g of sucrose [9]. The sucrose counteracts the occurrence of osmosis. Prior to the CSCs exposure to pulses from SPG1, YOPRO-1 dye (3 μM) was added to the CSCs solution. The permeabilization of cells membranes allows the dye to enter the CSCs cytoplasm and bind with nucleic acids (DNA RNA), thus the permeabilized CSCs will become fluorescent. YOPRO-1 emission at 510 nm has been detected using florescence microscopy. The percentage of cell population that becomes florescent indicates the success of the CSCs electropermeabilization. The complete protocol for CSCs preparation can be followed here [8].

Results

The previously mentioned artificial buffer was prepared to represent a 50 Ω load to aid impedance matching between the load and the generator. We preventively verified, performing trypan blue exclusion test, that CSCs remain alive in the buffer after more than three hours. Fig. 2 demonstrates that the buffer load is 50 Ω, as the waveform measured with the buffer is comparable to the waveform measured with a 50 Ω resistor. Fig. 2 depicts also the 100 ns, 200 ns and 300 ns pulse waveforms that were measured across the electroporation cuvette containing CSCs suspended with the 50 Ω, 0.3 S/m solution. These waveforms, shown in Fig. 2, illustrate the shape of pulses delivered to the CSCs during the primary permeabilization rate investigation. Our preliminary results indicate that exposing CSCs to 200 ns and 300 ns with 5 or more consecutive pulses results in >70% of the CSCs becoming permeabilized (green fluorescence in Fig. 3). Fig.3 indicates that 90% of the CSCs population is fluorescent and permeabilized when 20 consecutive 1.2 kV pulses, of 300 ns duration are delivered though the cuvette.

A principle of electropermeabilization is its non-thermal effect on the living organism. Therefore, we demonstrated computationally that the heating effect of the applied pulses on the CSCs buffer load with various SPG1 parameters is negligible. Following initial investigation that exposed CSCs to electric pulses, other buffer solutions of various conductivities where investigated (0.1 S/m, and 0.4 S/m) with pulse widths of 100 ns, 200 ns and 300 ns. Our results show that the SPG1 performance is insensitive of the load impedance, in the range between 10 Ω (0.4 S/m) to 60 Ω (0.1 S/m), demonstrating broadband matching performance (for pulses down to 100 ns) as far as the pulse waveform widths are considered. This effect shows good transition times preservation overall. However, the delivered pulse amplitude does change with the buffer impedance (data not shown).

Discussion and Conclusions

In conclusion, the overall system developed: the SPG1, the cuvette housing unit, and the buffer resulted in successful permeabilization of the CSCs with the possibility of real-time cell neutralization via non-thermal electropermeabilization. Promising results were obtained in terms of matching strategy and cell permeabilization to YOPRO-1 dye with various pulse durations.

The gathered results do not reflect whether the CSCs are dead or alive after electropulsation. Therefore, it is unknown whether they underwent reversible or irreversible electropermeabilization. Additionally, it is important to state that this does not mean that the CSCs exposed to 100 ns pulses, which resulted in a lower fluorescence, are not affected even if only poorly permeabilized. Viability studies are required to complement these preliminary experiments in the future.

The generator developed is well matched to the application and has low sensitivity to various types of solution filling the cuvette. The low sensitivity and range of programmable pulse regimes in the SPG1 is advantageous to the user which performs well in comparison to more generic, commercial sub-microsecond and nanosecond generators.

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Figures

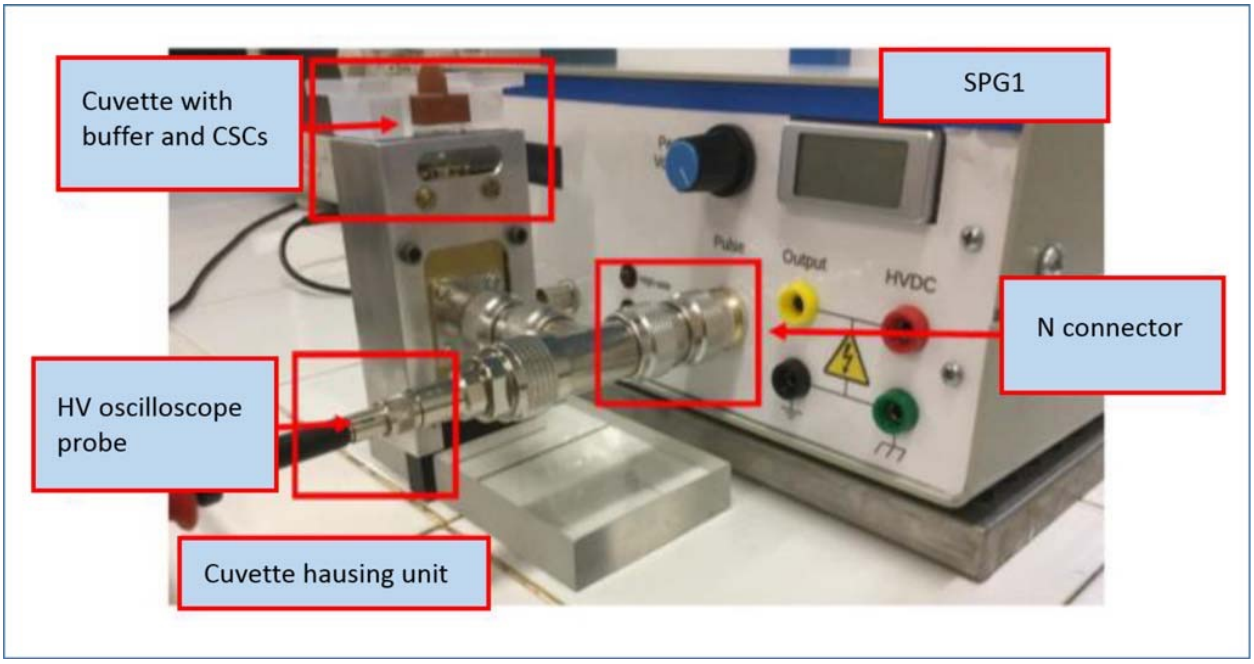

Figure 1. Instrumentation setup: the SPG1 generator, the cuvette housing unit filled with a 50 ohm buffer and CSCs

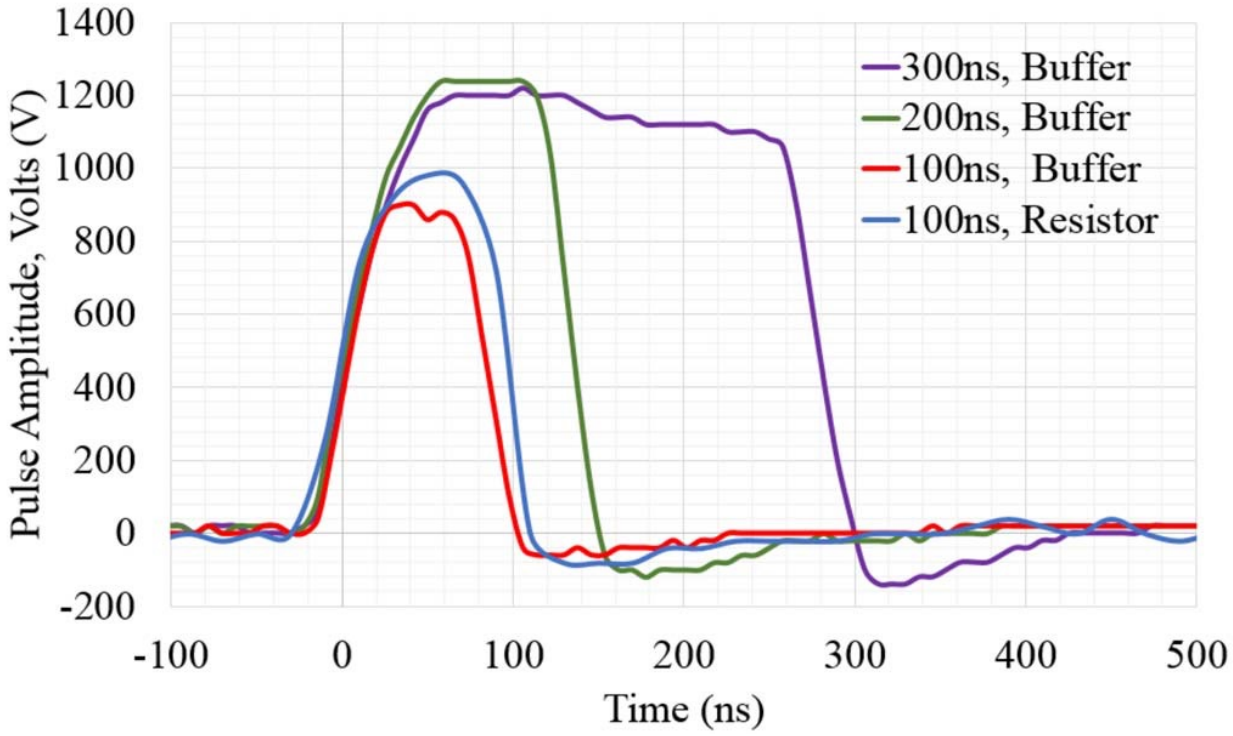

Figure 2. 100 ns, 50 Hz, 20 burst waveform, with 50 Ω resistor as SPG1 load (blue). And 100 ns (red), 200ns (green) and 300 ns (purple) pulse, 50 Hz, 20 bursts waveform measured with CSCs in the 50 Ω buffer load.

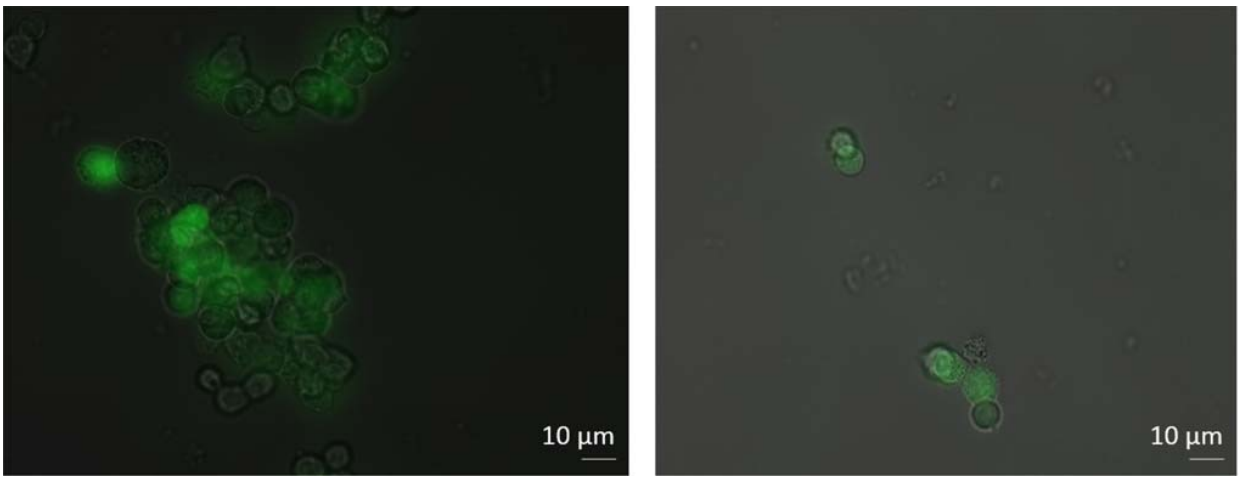

Figure 3. Example of permeabilized cells at 300 ns, ~1.2 MV/m, 20 pulses at 1Hz. Bright field (visible light) superimposed to fluorescence microscope images.