URSI GASS 2023, Sapporo, Japan, 19 – 26 August 2023

Microsecond electric pulses effects on stem cells: results from RISEUP project

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Abstract

Spinal Cord Injury (SCI), a major cause of paralysis, currently has no effective therapies. Every year almost 500.000 people are diagnosed with SCI worldwide. he difficulty on the neuronal restoration after SCI is based on the complex cascade of events that inexorably cause a degenerative chronic stage mainly favored by the non-permissive environment and limited capacity for axonal regrowth. RISEUP proposes to attain neuronal functional regeneration after SCI by an unprecedented and unique bio-hybrid-compatible electro-activated and wireless rechargeable implantable technology. RISEUP introduces high voltage microsecond electric pulses stimulations and low amplitude direct currents on a combination of stem cells (induced neural stem cells and multipotent stromal cells), whose transplantation is facilitated by an innovative scaffold biomaterial.

Here the first results from RISEUP projects on microsecond electric pulses effects on stem cells are reported.

1 Introduction

SCI is a neurological and pathological state characterized by motor, sensory and autonomic dysfunctions, which affects around 500,000 people every year [1]. So far, several therapeutic strategies based also on stem cell transplantation have been studied, but the results are poor and an effective protocol for spinal cord regeneration is still missing.

RISEUP project (n. 964562), funded by the European community in the H2020 FET-OPEN program, provides for the development of an innovative system for the regeneration of SCI based on the transplantation and the microsecond (µsec) electric pulses stimulation, through a biocompatible, biodegradable, and electrified support, of mesenchymal (MSCs) and induced neuronal (iNSCs) stem cells. The hypothesis is that, by modulating the calcium fluxes [2, 3] of iNSCs through µsec pulses stimulation, these cells will differentiate in mature neurons acquiring the ability to regenerate the lesioned area. Moreover, MSCs stimulation could induce their differentiation in neuronal-like cells, able to release neurotrophic factors necessary for neurons survival.

In this paper we show the effects induced on both kind of stem cells by µsec stimulation.

2 Methods

2.1 Exposure conditions

Cells (350.000 iNSC and 100.000.MSC) were plated 48 hours before exposure in 10 cm Petri dishes supplied with a Polydimethylsiloxane (PDMS) pool to enable the inserting of two titanium electrodes placed at 1 cm distance.

Just before pulses delivery, the medium inside the electrodes is replaced in order to have a volume of medium of 2 ml maximum.

The pulses generator used for these experiments was supplied by Leroy Biontech.

The pulses stimulation condition was:

- Bipolar pulse: **100 µs + 100 µs**
- Intensity: **250 V/cm**
- 2 pulses / min
- Duration: 30 min

All the biological analysis were performed 24 hours after the exposure.

2.2 Cell proliferation and viability

To estimate the proliferation and to contemporary evaluate the viability, cells were counted through Burker's chamber,

after the staining with erythrosine B, a dye who detects dead cells

2.3 Cell cycle analysis

Adherent cells were harvested by trypsinization and collected with floating ones; the pool was washed twice in PBS, then fixed in ice-cold ethanol 80% (1×10^6 cells/ml) overnight. An aliquot of the suspension (at least 5×10^5 cells) was then washed twice in PBS and stained with PI (50 µg/ml) in a mix containing RNAse A (50 µg/ml), Triton X-100 (0.1%), and EDTA (0,1 mM) in PBS, in the dark, for 60 min at room temperature, then immediately analyzed. A FACScan flow cytometer (Becton Dickinson, Bedford, MA, USA), equipped with a 488-nm argon laser, was used for the flow cytometric analyses. The evaluation of cell cycle distribution by DNA content analysis was performed by the FlowJo software.

2.4 Gene expression

Total RNA was extracted with Direct-zol RNA Miniprep (Zymo research) and 500 ng of RNA were converted in cDNA by TaqMan reverse Transcription Reagent (Applied Biosystem) according to manufacturer's indications. 25 nanograms of cDNA were amplified with specific primers using SYBR Green master mix (Thermo Fisher Scientific) and a 7500 Real-Time PCR system (Thermo Fisher Scientific).

3 Results

Microsecond electric pulses stimulation did not affect cell proliferation and survival of both iNSCs and MSCs (Figure 3), nor any phase of the cell cycle.

About gene expression, the eventual variation of markers of cell stemness, proliferation, differentiation and apoptosis, was investigated. iNSCs did not show any modulation of the considered genes; while in MSCs a slight, but significant, increase of *sox2* expression was observed.

4 Conclusions

The stimulation protocol described here is the first we used in RISEUP project, in order to investigate the response to µsec pulses of iNSCs, that, as far as we know, have never been subjected to this kind of stimulation before. Currently, new stimulation protocols aimed at inducing stem cells differentiation are under investigation

5 Figures

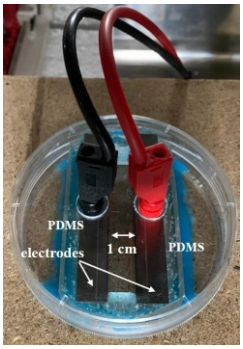

Figure 1. Exposure setup. PDMS "pools" are placed inside a 10 cm petri dish, to enable the insertion of 2 titanium electrodes implanted at 1 cm distance. Cells are plated inside the electrodes

Figure 2. Leroy Biontech **p**ulses generator

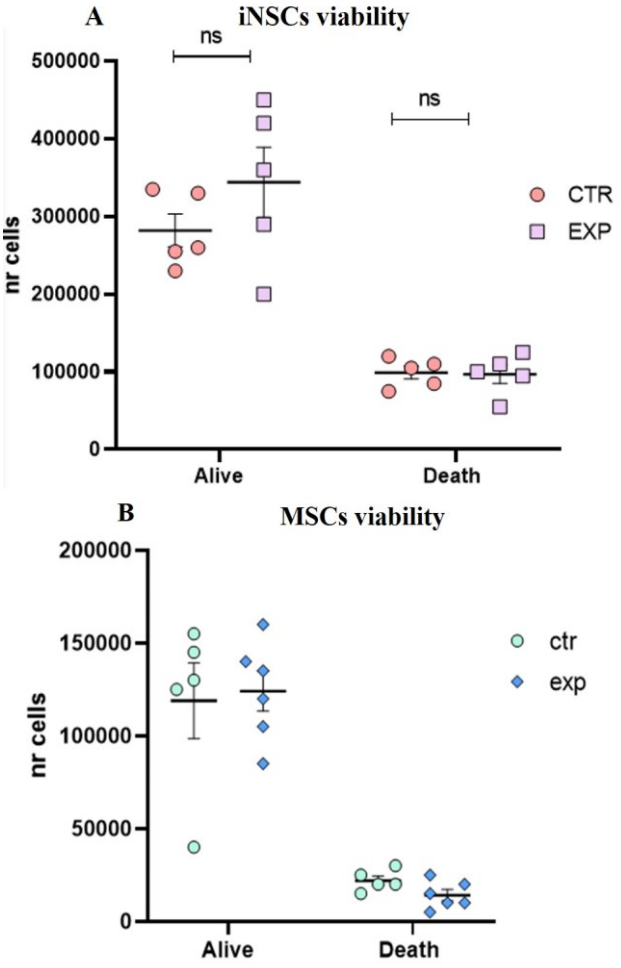

Figure 3. **iNSCs (A) and MSCs (B) viability**

Acknowledgements

This project is supported by FET-OPEN H2020 grant. Grant number 964562

References

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