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Analysis And Control Of Calcium Oscillations In Differentiating Mesenchymal Stem Cells Using Pulsed Electric Fields

Leslie A. Vallet [1], Franck M. André [1] and Lluis M. Mir [1]
[1] Université Paris-Saclay, Institut Gustave Roussy, CNRS, Metabolic and systemic aspects of oncogenesis (METSY), 94805, Villejuif, France

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Abstract

Mesenchymal Stem Cells (MSCs) are adult stem cells able to give rise to many cell types such as osteoblasts, adipocytes or chondrocytes. These last decades, a high interest has grown around the development of clinical applications using MSCs. In another respect, calcium is a ubiquitous secondary cell messenger, encoding important information for the cells, for instance in the form of oscillations. It has been shown that MSCs naturally exhibit spontaneous calcium (Ca^{2+}) oscillations, whose frequency is varying over the course of differentiation processes. Subsequently, we aim to assess whether, by manipulating the frequency of Ca^{2+} oscillations, we might influence proliferation or differentiation events in MSCs. In the purpose to achieve control over Ca^{2+} oscillations, we exposed MSCs to short high voltage pulsed electric fields (PEFs) capable of permeabilizing the cell membrane to Ca^{2+} ions present in the surrounding medium, eliciting a Ca^{2+} response similar to the natural oscillations.

1 Introduction

Mesenchymal Stem Cells (MSCs) are adult stem cells able to differentiate into various cell types constituting connective tissues in the body, such as osteoblasts, adipocytes or chondrocytes, but also, according to some more recent studies into other cell types, such as muscle cells or neuron-like cells. It has been observed that these cells naturally exhibit spontaneous Ca^{2+} oscillations [1] whose frequency varies over the course of differentiation processes. Ca^{2+} is an important cellular secondary messenger and variations in the frequency and/or in the amplitude of Ca^{2+} oscillations can be observed in response to stimuli (chemical/physical, physiological/or not) [2]. In our case, we are interested in the changes in Ca^{2+} oscillations that might be triggered by stimuli inducing cellular proliferation or differentiation events. Downstream of these stimuli, Ca^{2+} oscillation frequency and/or amplitude can embed important information, subsequently decoded by some proteins in the cell whose activity is Ca^{2+}-sensitive [2]. Thus, we wonder whether taking the control over Ca^{2+} oscillations frequency and/or amplitude might mimic the effects of the physiological stimuli, bypassing them by directly triggering the Ca^{2+} response.

Therefore, the question was how to control Ca^{2+} oscillation frequency and/or amplitude. Previous studies in our laboratory have shown that applying high amplitude ultrashort electric pulses able to permeabilize the cell membrane to Ca^{2+} ions can induce Ca^{2+} oscillations similar to the naturally observed ones (in shape, amplitude and duration), due to the Ca^{2+}-Induced Ca^{2+} Release response followed by elimination of cytosolic Ca^{2+} excess by pumps located at the cell and endoplasmic reticulum membranes [3]. Thus, we used this technology in this study, using exposure chambers which allow to perform long-term exposures to pulsed electric fields (PEFs) in regular cell culture conditions.

2 Material and methods

Cells: MSCs were grown in Dulbecco Modified Eagle Medium supplemented with 10% Foetal bovine serum and antibiotics (complete DMEM), and incubated at 37°C in a humidified incubator with 5% CO_2. Cells were induced in adipogenic differentiation with an induction medium composed of complete DMEM supplemented with dexamethasone (1 µM), indomethacin (200 µM), IBMX (500 µM) and insulin (10 µg/mL) used in alternance with a maintenance medium composed of complete DMEM with insulin only.

Ca^{2+} oscillations: monitoring was performed using time lapse microscopy recording for 15 min with 1 picture recorded every 10 sec as previously described [3]. Ca^{2+} oscillations frequencies were determined by means of sequential treatment of these recordings with CellProfiler image analysis software and a Matlab program. The PEFs generator was built at Univ. of Zaragoza, Spain. It delivers impulsions whose duration ranges from 1 to 100 µs. The output voltage can reach 1000 V.

3 Results and discussion

3.1 Monitoring the evolution of calcium oscillation frequencies over the course of adipogenic differentiation of MSCs

The analysis of Ca^{2+} oscillations frequencies at various time points of the differentiation allowed us to identify changes occurring from the very early phases of the differentiation to the end of the process when the cells are fully differentiated. For instance, at the end of the differentiation process, most of the MSCs induced in adipogenic differentiation did not display the Ca^{2+} oscillations (representing 65% of the cells) of the non-differentiated MSCs (figure 1). Of note, the cells that were still oscillating after 22 days of adipogenic differentiation were the ones that did not exhibit adipocyte morphological features (data not shown).

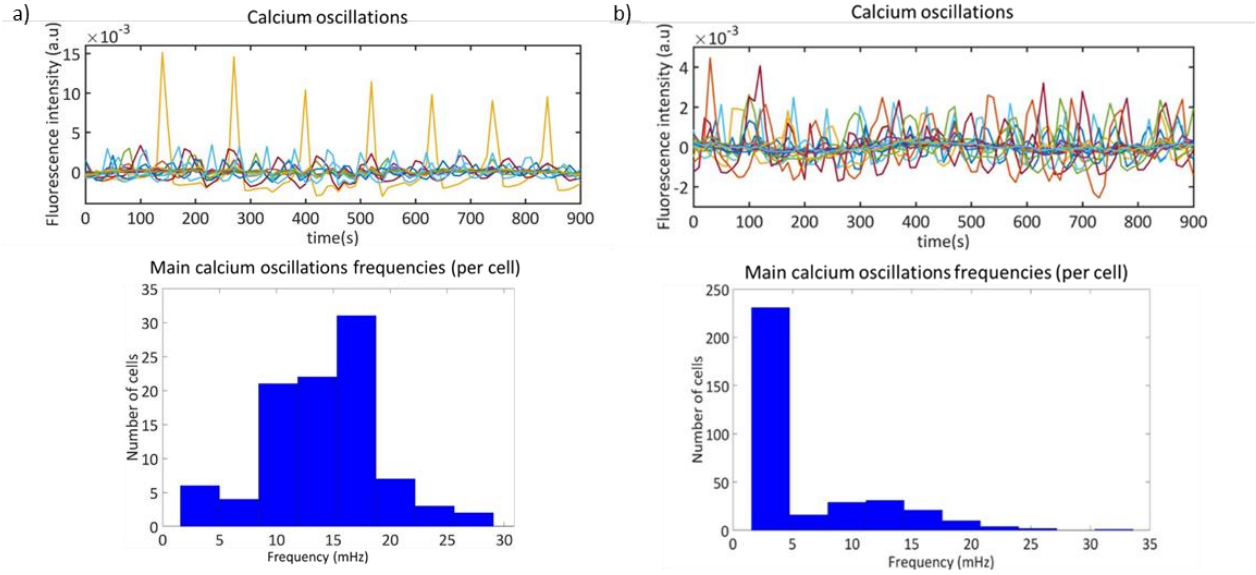

Figure 1: Ca^{2+} oscillations of MSCs a) non-differentiated, in standard culture conditions. b) induced in adipogenic differentiation for 22 days. Upper panels show data on 20 cells for clarity of the images. Lower panels show data on 129 and 345 cells for a) and b), respectively.

3.2 MSCs exposure to Pulsed Electric Fields

In a second step, our study is aiming at taking the control over Ca^{2+} oscillations by using pulsed electric fields permeabilizing MSCs membranes to Ca^{2+} ions present in the surrounding culture medium. For now, our work in this part of the project has more focused on optimizing the exposure conditions (electrode material, pulse duration, exposure chamber design), overcoming step by step technical limitations due to the "long-term" aspect of exposures to PEFs required in our experiments. In the latest designed configuration, PEFs are delivered with plate grade 2 titanium electrodes sets, arranged on both sides of a culture well chamber.

4 Conclusion

We aim to modify biological cells physiology by electric means, namely, to control cells differentiation through the delivery of short and intense PEFs for long periods. In our efforts to achieve our goal, we have found physical, chemical and biological limitations that have been overcome step by step. The numerical methods for quick analysis of the Ca^{2+} oscillations in a large number of cells have been developed. The methods and set-up for the exposure of the cells for weeks are now set and the cell physiological reactions can be studied.

References

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