COST EMF - MED (Action BM1309):
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STSM Report:

Cell Electroporation using EMF Signals

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Abstract:

Electropermeabilization is a process in which intense short electric pulses are applied to cells in order to produce structural changes in the plasma membrane. Fluorescent dyes that do not cross the membrane (unless the membrane is transiently or permanently permeabilized) are markers often used to detect cell membrane permeabilization. The objective of this STSM was to characterize the level of electric pulse that is sufficient to permeabilize the plasma membrane to YO-PRO-1 and to determine if nuclear or cytoplasm staining occurs after cell electropermeabilization in human primary mesenchymal stem cells – haMSC. In vitro experiments were performed in order to test haMSC permeabilization to YO-PRO-1 after the application of one single electric pulse of 100 µs duration.

A. Purpose of the STSM

The aims of this STSM were: (i) to understand the cell staining when using the fluorescent non-permeant YoPro1 dye after the application of one pulse in 100 µs duration and at about 1 kV/cm field magnitude (staining of the nucleus, or of the cytoplasm, or of the membrane?) (ii) to identify the minimum level of electric field that causes nuclear staining (iii) to determine the minimum concentration of YoPro1 that can stain the nucleus of the cell. This clarification is a first step towards ulterior studies on haMSC and other cells electropermeabilisation. Another aim of this STSM was to learn about the electroporation fundamentals and the methods to analyse cell electroporation (in particular classical and confocal fluorescence microscopy) used in the host institution.

B. Work Description

The main goal of this STSM mission was to investigate the lowest levels of the field amplitude of the electric pulse that can permeabilize the human primary mesenchymal stem cells to the Yo-Pro-1 and let the staining go inside the cell through in vitro experiments. With this aim, I should thank Dr. Tomas Garcia-Sanchez, Post-Doc Researcher and Dr. Franck Andre, Senior Researcher in the host institution for their tutorials in setting up the experiment conditions.

We analyzed the staining process by using florescent and confocal microscopy. The Yo-Pro-1 stains the nucleic acids but in most of the published articles, photographs seem to indicate a nuclear staining. In the host institution, classical fluorescent microscopy seemed to display also extranuclear Yo-Pro-1 staining. The aim of the STSM, apart from the learning on cell fluorescence fundamentals and methods, was to analyze this extranuclear staining. Therefore, in order to identify the stained part of the cell, cell compartments were stained also with other specific markers: Hoechst, blue fluorescent dye that only stains DNA (thus the nucleus) and Rhodamine B a red fluorescent dye that stains the cell cytoplasm. After several experiments, Dr. Andre suggested to use Wheat Germ Agglutinin Alexa Fluor 594 conjugate, a bright, red fluorescent dye that stains the membrane of the cells in order to have detailed image instead of Rodamine B.

We observed the kinetics of Yo-Pro-1 penetration into the electropermeabilized cells, during 30 minutes after the pulse in classical epifluorescence microscopy (Zeiss AxioVert 100, Germany) and during 5 minutes in confocal microscopy (Leica SP8).

The experimental setup consisted of a cliniporator (IGEA, Italy) for the delivery of the pulses to the cells and of microelectrodes (300 µm for the interelectrodes distance) (Figure 1) and an attenuator (3 times attenuation). We applied pulse electric fields of 130 V for 1400 V/cm, 90 V for 1000 V/cm, 76 V for 800 V/cm and 60 V 1 pulse to the cells.

Three concentrations of the Yo-Pro-1 solution in SHM Buffer were tested (0.1, 2 and 5 µM) in order to identify if and how the concentration of Yo-Pro-1 may affects the staining of the various cell compartments. The haMSC were cultured on glass coverslips (11mm x 32 mm) with approximately 150.000 cells per coverslip at the time of the treatment.

Hoechst was applied to the cells one hour before the experiments. Cells were stained with Wheat Germ Agglutinin Alexa Fluor 594 conjugate 10 minutes prior to the experiments. Just before the pulse was delivered, Yo-Pro-1 was added to the cells. Coverslips placed on microelectrodes were monitored in real-time under microscope. In addition to the electroporation groups, we tested the control groups for all concentrations of Yo-Pro-1.

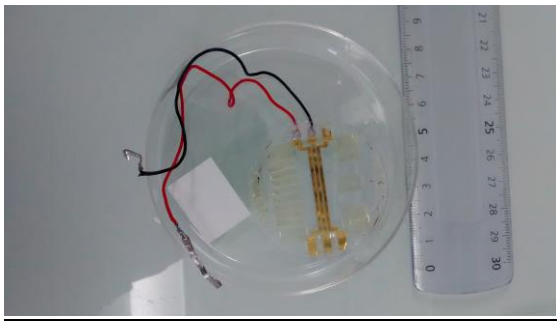

Figure 1: Microelectrodes (300 µm for the interelectrodes distance)

C. Results

The results revealed that human mesenchymal stem cells can be permeabilized by the application of one single, high field intensity pulsed electric field (Fig 1). Interestingly, at the lowest field amplitudes applied, the Yo-Pro-1 green fluorescence is not more intense in the nucleus than in the cytosol, particularly at low external Yo-Pro-1 concentrations. The increase in the field amplitude resulted in a clear nuclear staining, more intense than the cytoplasmic one. The confocal microscopy showed that the cytoplasmic labeling was localized inside the cell and not at the membrane level. So, in agreement with the physicochemistry of this dye, the cytoplasmic staining should correspond to Yo-Pro-1 interactions with the RNA molecules, abundant in the cytosol, and not to an interaction with the membranes. Since, very often, cell permeabilization is monitored following the nuclear

staining, the thresholds of field amplitude determined with Yo-Pro-1 should be lowered because the cytoplasmic staining is actually recordable at lower field amplitudes and/or lower external Yo-Pro-1 concentrations.

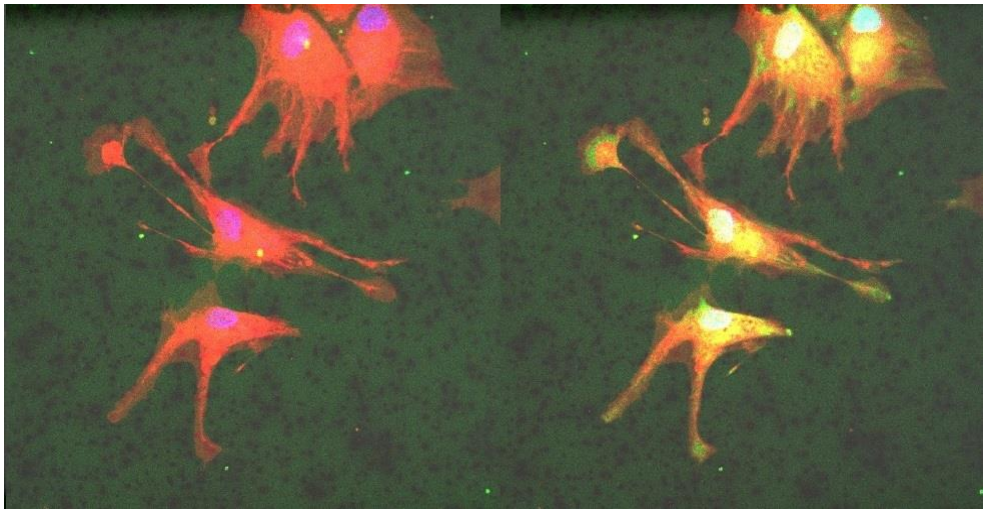

Figure 2: Confocal microscopy images just after and 5 minutes after the delivery of one electric pulse of 1000 V/cm in the presence of 2 µM YoPro1. Before the pulse delivery cells were stained with Rhodamine B and Hoechst for 1 hour.

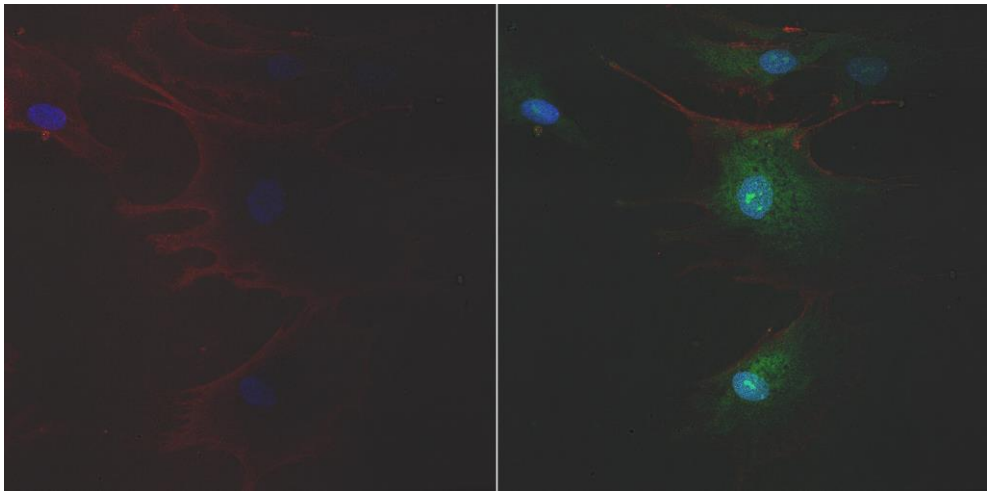

Figure 3: Confocal microscopy images just after and 5 minutes after the delivery of one electric pulse of 600 V/cm in the presence of 2 µM YoPro1. Cells were stained with Hoechst for 1 hour and Wheat Germ Agglutinin Alexa Fluor 594 conjugate 10 minutes before the pulse delivery.

D. Future collaboration with the host institution

During the stay in the host institution, we discussed future long-term projects between the Biophysics Department of Gazi University, Medical Faculty and the host laboratory, including the possibility of a later visit to the host institution for a sabbatical period of one year. We decided to continue these experiments. In addition, we planned to study calcium oscillations of cells during my sabbatical period.

E. Expected Publications

Further studies are needed in order for the results of this preliminary experiment to be published. In particular, the kinetics of the cytosolic and nuclear staining of haMSCs should be explored in detail as a function of the electric pulse field intensity and as a function of the Yo-Pro-1 external concentrations. We have already planned to improve these experiments during a longer period of stay as part of our future collaboration.

Confirmation by the host institution of the successful execution of the STSM:

We confirm that Elcin Ozgur-Buyukatalay has performed the research work as described above.

Contact Person of Host Institution

Lluis M. Mir

Name of researcher

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