**Effects of a Nuclear Permeabilizing High Voltage Micropulse on Nucleus of Living Cells *in vitro***

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**INTRODUCTION**

Conventional electropermeabilization, employing long lasting pulses and low electric fields, is widely used to permeabilize the plasma membrane and allow the entry of molecules into the cytoplasm of living cells while leaving intact organelles [1]. Previous studies suggested that the application of high electric fields with ultra short duration allowed permeabilizing the membranes of intracellular organelles like the nuclear membrane [2], [3]. Intense pulsed electric fields could enhance gene delivery to the nucleus [3].

In the present study, we focused on the modifications induced by electric fields on nuclear morphology and permeabilization of CHO (Chinese Hamster Ovarian) cells.

**METHODS**

Cells were cultured over night at 37°C on labtek slides for adhesion and, the following day, were electropermeabilized in a pulsing buffer (10 mM Phosphate Buffer, 250 mM sucrose, 1 mM MgCl2) with a GHT electropulsator. The permeabilization efficiency was followed with use of propidium iodide (PI, 0.1 mM). In the same time, cells were imaged by fluorescence on an inverted digitized videomicroscope with a 63X objective (Leica, Roper camera, Metavue software).

For the double pulsation procedure we used the following protocol: 0.7kV.cm-1, 5 ms, 1Hz, X10 and 2 min later a single 5kV.cm-1, 5 µs pulse. We acquired an image before the procedure, every 20 s after the first pulse train and every 1 min during 10 min after the second pulse condition. By image processing (Optimas 6.5), we calculated the changes of nucleus area and mean fluorescence during the procedure.

**RESULTS**

The electropulsation induced membrane permeabilization brought an enhancement of the fluorescence in the nucleus. The first train of pulses induced a slight swelling of cells nucleus (Fig 1). The mean fluorescence of the nucleus was increased and reached a saturation level in less than 2 minutes (Fig 2). This level was maintained along the 10 following minutes when the nucleus slowly recovered its initial size (Fig 1 & 2).

The application of a second high voltage pulse did not significantly affect the enhanced mean fluorescence of the nucleus but delayed its return to its initial size (Fig 1 & 2).

**CONCLUSIONS**

The electropulsation induced nuclear swelling reflects a perturbation of the nuclear envelope. This can be a direct or a by effect of the pulse [4]. The second pulse apparently “stabilizes” this perturbation. The PI fluorescence results suggest that the chromatin structure is not significantly affected.



**Figure 1**: Nucleus area changes (%) during permeabilized state



**Figure 2** : Mean fluorescence (au) of the nucleus during permeabilized state

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