ELECTRONIC SUPPLEMENTARY INFORMATIONS

Materials and methods

Cell culture.

Chinese hamster ovary (CHO) cells were used. The WTT clone was selected for its ability to grow in suspension or plated on Petri dishes. They were grown as previously described in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5 % foetal calf serum.

Cells were plated at a density of $1x10^6$ cells per petri dish (35mm, Nunc). After 24 hours of culture, cells were confluent giving a 2D pseudo tissue with an intercellular matrix (figure S1 A).

Electropulsation apparatus.

Electropulsation was operated by using a CNRS cell electropulsator (Jouan, St Herblain, France) which delivered square-wave electric pulses. An oscilloscope (Enertec, St. Etienne, France) monitored pulse shape. Two stainless-steel parallel rod electrodes (0.95mm diameter) are connected to the voltage generator. The distance between the electrodes was 6.5 mm (Figure S1 B)

Electropermeabilization procedures.

Penetration of propidium iodide (100 µM in a low ionic strength pulsation buffer (10 mM phosphate, 1 mM $MgCl_2$, 250 mM sucrose, pH 7.4)) was used to monitor permeabilization. Cells were washed and incubated in 500 µl of the PI containing pulsing buffer. The electrodes were placed in contact with the dish that was covered by the cell monolayer. 8 (or less) pulses lasting 100µs at a frequency of 1 Hz were applied at a given voltage at room temperature (figure S1 C). PI entrance into the cell after electropermeabilization led to an increase of the fluorescence intensity when PI reached the nuclei of the cells.

Visualization of the permeabilization efficiency

After pulsation, cells were placed onto the stage of an epifluorescence stereomicroscope (Leica MZFL III) for visualization. This set-up allowed a broad observation, larger than the inter-electrode distance. High magnification digitized images (12 bits 1.3 M pixels image) were obtained by using a cooled CCD camera (Roper Coolsnap fx). Fluorescent cells were observed using the x 0.8 magnification (Figure S1 D).

The MetaVue software (Universal, USA) drove the camera from a Dell computer. The exposure time was set at 1 s. with no binning. The fluorescence excitation was obtained with a Mercury Arc lamp (HBO, Osram, Germany) and the G filter set (Leica).

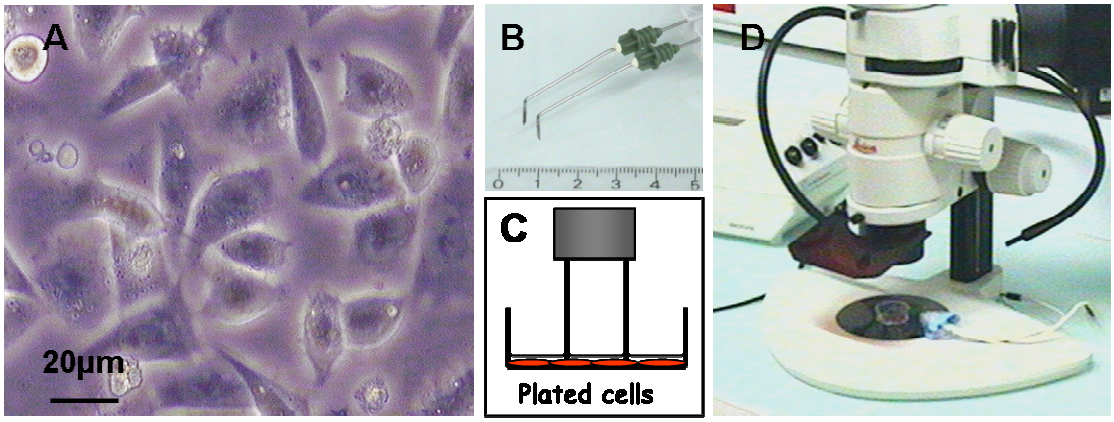

Figure S1

Fluorescence measurements

The PI fluorescence intensity was correlated to the level of permeabilization, PI emission being detectable only when the probe was in the cell cytoplasm. The image in absence of electric field (0V) was subtracted from the image at the given electric field condition (325 V in figure S2) in order to eliminate the background signal (figure S2). Then a low pass filter to smooth the result image and a pseudo color LUT were applied in order to better visualize the threshold of permeabilization. The area of the region of interest (ROI) above the permeabilization threshold (white area) and the mean fluorescence in this ROI were quantitatively evaluated (Measure/region measurement). Data were then transferred for statistical analysis (Excel 7 software).

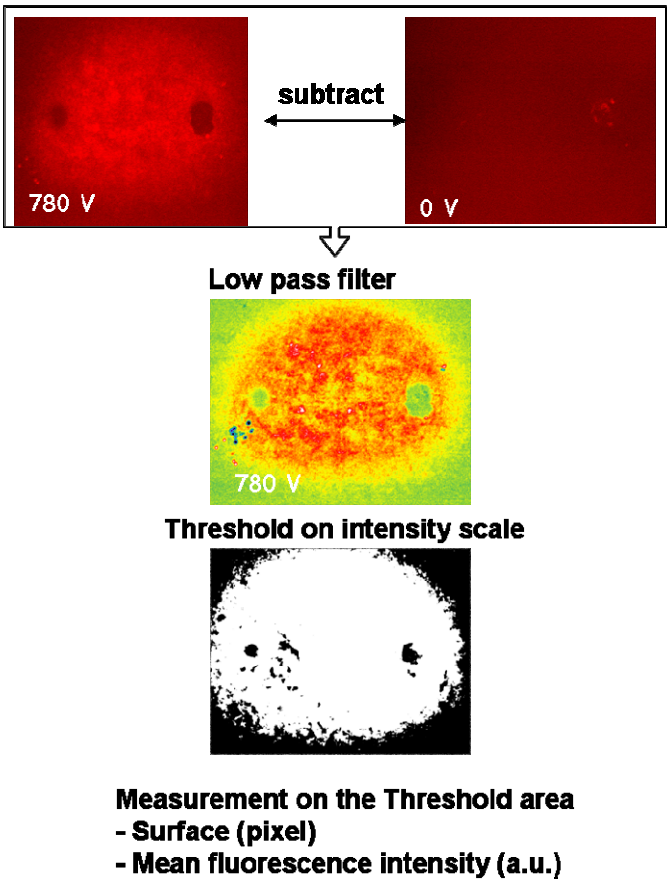

Figure S2

Statistical analysis

For each condition, the mean of 3 to 4 independent experiments (mean+/- sd) was shown.

Figure S1: Experimental set-up

A- A CHO cell monolayer under phase contrast observation. Scale bar is 20µm. B- Needle electrode set. Inter-electrode distance is 6.5mm. C – Drawing of the electropulsation protocol. D- Digitized Stereomicroscope used for visualization.

Figure S2: Data processing

The image in absence of electric field (0V) was subtracted from the image under the given electric field condition (780V) in order to eliminate the background fluorescence and heterogeneity of the light beam excitation. On the differential image, a low pass filter and a

pseudo color LUT were applied in order to better visualize the permeabilization (i.e. the PI fluorescence levels). The area of the region of interest (ROI) above the permeabilization threshold (white area) and the mean fluorescence in this ROI were quantitatively evaluated.