Single-Cell Electroporation Using a Multifunctional Pipette

Alar Ainla, Shijun Xu, Nicolas Sanchez, Gavin D. M. Jeffries and Aldo Jesorka

Electronic Supplementary Information (ESI)

Table of contents

Supplementary Methods

Method S1. Cell preparation

Adherent NG-108-15 cells were cultured in thin glass bottom (#1 coverslip) petri dishes, suitable for confocal imaging, for 2-6 days in Dulbecco's modified eagle medium (DMEM) supplemented with fetal calf serum (10%).

Method S2. Dye loading of cells

The cells were loaded with the calcium sensitive dye, Calcium-Green™-1 AM ester (Life Technologies Inc, Carlsbad, USA). A 200 µM stock solution was first prepared using DMSO, which was then diluted with Ringer's solution (VWR) to obtain a final working concentration of 4 µM. After removing the petri dish from the incubator, the culture medium was removed and the cells were rinsed twice with PBS buffer, followed by the addition of 2ml of the calcium dye loading solution. The cells were exposed to the dye loading solution for 1 h at room temperature, then rinsed twice with phosphate buffered saline (PBS) buffer

(Ca^{2+} free). This buffer had an ionic strength of 60 mM; containing 5 mM Trizma base, 30 mM K_3PO_4, 30 mM KH_2PO_4 and 0.5 mM NaEDTA, NaOH adjusted to pH 7.8, and was also used as the main buffer for all experiments.

Method S3. Preparation of carbon fiber electrodes

Plastic encapsulated ProCFE carbon fibers, with an OD 5 µm, were obtained from Dagan Corp. and assembled as follows: An OD 1.0 mm borosilicate glass tube was trimmed to 5 cm in length, using a ceramic glass cutter or diamond scribe. A 10 cm long silver wire was inserted such that 1cm protruded from the tube. Conductive silver epoxy (ELFA Electronics, Sweden) was mixed in a 1:1 ratio and a drop of 2-3 mm diameter was applied to the protruding end. The glass rod with the epoxy covered end was quickly inserted into the rear of the plastic covered carbon fiber, such that the epoxy filled the plastic barrel completely. Additional epoxy was applied where necessary, to achieve a complete seal. The carbon fibers were stored vertically, with the tip pointing downwards, for at least 1h before use, to ensure that the silver epoxy had sufficiently hardened.

Method S4. Electric field simulation

Electric field strengths were evaluated in numeric simulations using COMSOL Multiphysics 4.1 employing the electric currents (ec) model. The electrical conductivity of water was set to an initial value of $\sigma = 5.5 \cdot 10^{-6}\, S/m$ (pure water in COMSOL). Our model is, however, insensitive to the exact values of electrical conductivity, as long as the value is used consistently throughout the calculations. Two simulation series were performed: one for the carbon fiber electrode, and another for the integrated Field's metal electrode, using the parameters outlined in tables S1 and S2, respectively. In both cases the "Cell" was modeled to be 5 µm high, a diameter of 20 µm, with an insulating membrane and a conducting interior.

Method S5. Fabrication of the Pipette with an integrated electrode

PDMS multifunctional pipettes were fabricated as described earlier[21]. Electrodes were prepared as shown in Figure 1. First, the bottom of the well connecting to the electrode channel was filled with grains of Field's metal (Alloy MCP61, Mindsets Ltd, Waltham Cross, UK). Thereafter, the pipette was heated to ~70-90 ˚C for 1min on a hot plate. Pressure of up to 2.7 bar was then applied, which caused the molten metal to quickly (<5s) fill the channel, forming a droplet at the pipette tip. The pipette was removed from the hot plate, and left to cool to room temperature. Excess metal was removed from the tip using tweezers. The pipette was inspected under a light microscope to check for air entrapped in the metal filling. Defects were corrected by repeating the filling cycle.

Gold coating. In order to eliminate electrochemical degradation of the Field's metal, we electrodeposited a gold film onto the exposed electrode surface at the pipette tip. For this purpose the tip was immersed into a common commercial $KAu(CN)_2$ based gold plating solution A3000 (Aurotech, Sweden) with Na_2CO_3 added for pH adjustment. A Pt wire was used as a counter electrode and a potential of -1.6V (relative to the Pt wire) was applied for 10 min to the Field's metal electrode for deposition.

Interfacing. A custom pipette holder was ordered from Teadusmosaiik OÜ (Tartu, Estonia). This holder was equipped with M4 screw holes at the top surface of the manifold, which allowed access to the pipette wells. The holes are normally hermetically sealed with Teflon tape tightened screws. The electrode-fitted well was interfaced with a spring loaded battery contact (ordered from ELFA, Sweden), which was fitted into a Nylon™ screw. The spring-loaded contacts ensured good electrical connection without putting strain on the Field's metal plug.

Table S1. Simulation settings for the external carbon fiber electrode

Parameter	Values
Outer dimensions of the simulation volume	100 µm x 100 µm
Height of the simulation volume	50 µm
Radius of the fiber tip	2.5 µm
Height of the fiber (center)	2.5 µm
Distance between fiber and center of the cell (series)	10, 15, 20, 25, 30, 35, 40 µm
Calculated electrical resistance of the simulation volume (R_{sim})	16 GΩ

Table S2. Simulation settings for the integrated Field's metal electrode

Parameter	Values
Bottom dimensions of the simulation volume	300 µm x 200 µm
Height of the simulation volume	150 µm
Width of the pipette tip	200 µm
Electrode size	20 µm x 20 µm
Thickness of the bottom membrane (bottom to electrode distance)	20 µm
Pipette height (distance between pipette bottom and surface) (series)	0, 5, 10, 20, 40 µm
Pipette angle	30˚
Pipette position in the middle of sim. volume	
Distance between pipette and center of the cell (series)	10, 20, 30, 40, 50, 60, 80, 100 µm
Calculated electrical resistance of the simulation volume (R_{sim})	9.1 GΩ

The total resistance of the electroporation setup can be calculated as a sum of resistances of electrodes R_e, the interface between the metal and the liquid R_{int} , and the liquid bath R_B. In this particular geometry we can divide the resistance of the liquid bath further into serial components. $R_B = R_{sim} + R_{B1} + R_{B2} + R_{B3}$. R_{sim} describes the resistance of the simulation volume, which is in the immediate vicinity of the electrode, R_{B1} describes a spherical extension of this volume extending to the surface of the bath, R_{B2} describes the resistance of the bath as a thin disk shaped water layer ($r \gg h$), and finally, R_{B3} describes the resistance around the cylindrical gold counter electrode, which is placed as a loop inside the liquid bath. The geometry of the setup and all resistances are shown in figure S1.

The resistances between the spherical surfaces can be calculated from the equation,

$$R = \frac{\rho}{4\pi}\left(\frac{1}{r_1} - \frac{1}{r_2}\right)$$

While for cylindrical and disk shaped volumes it is expressed as,

$$R = \frac{\rho}{2\pi L} ln \frac{r_2}{r_1}$$

where $\rho = 1/\sigma \approx 1.8 \cdot 10^5 \Omega \cdot m$ stands for the resistivity of water. The geometries of the macroscopic parts are given in table S3.

Table S3. Geometries of the macroscopic parts of the electroporation setup

Parameter	Symbol	Value
Radius of the dish	r	22 mm
Height of water level in the dish	h	~4 mm
Length of Au wire	$l = 2\pi r$	~140 mm
Diameter of Au wire	D_{gw}	0.2 5mm
Radius of simulation volume (Carbon fiber electrode)	r_s	50 µm
Radius of simulation volume (Integrated electrode)	r_s	150 µm

In case of the integrated electrode, the geometries of the macroscopic parts remain the same, only the simulation volume changes. The resistance of each component can be calculated for both setups (Table S4).

Table S4. Serial resistance components.

This estimation of resistances justifies the assumption that the entire voltage drop in the bath occurs inside the simulation volume. The error in both case should be < 4 %.

Component	Expression	Value (Carbon fiber)	Value (Integrated electrode)
$\boldsymbol{R_e}$	$measured\ exp.$	--	~40 Ω
$\boldsymbol{R_{int}}$	$unknown$	--	--
$\boldsymbol{R_{sim}}$	$simulation$	16 GΩ	9.1 GΩ
$\boldsymbol{R_{B1}}$	$\frac{\rho}{2\pi}\left(\frac{1}{r_s}-\frac{1}{h}\right)$	0.57 GΩ	0.18 GΩ
$\boldsymbol{R_{B2}}$	$\frac{\rho}{2\pi h} ln \frac{r}{h}$	12 MΩ	12 MΩ
$\boldsymbol{R_{B3}}$	$\frac{\rho}{2\pi l} ln \frac{h}{D_{gw}}$	0.57 MΩ	0.57 MΩ

The actual electric conductivity of the buffer solution can be estimated from the conductivity equation for strong electrolytes (*Atkins, Physical Chemistry 6th ed, 2000*).

$$\Lambda = \sum_i v_i \lambda_i$$

Where v denotes the concentration of a certain ion and λ this ion's molar conductivity. In case of ECB solution, the most prominent ions are Na^+ (~140 mM, $\lambda = 5.01\ mS\ m^2\ mol^{-1}$) and Cl^- (~140 mM, $\lambda = 7.63\ mS\ m^2\ mol^{-1}$), giving $\Lambda \approx 1.8\ S\ m^{-1}$, which is ~330,000 times larger than for MilliQ (18 MΩ) water, giving a resistance estimate of the liquid bath to be ~50 kΩ in the case of the carbon fiber system, and ~28 kΩ in the case of the integrated electrode. These values are still significantly larger than, for example, the electrode resistance, which can be neglected.

Figure S1. A schematic representation of the electrode setup

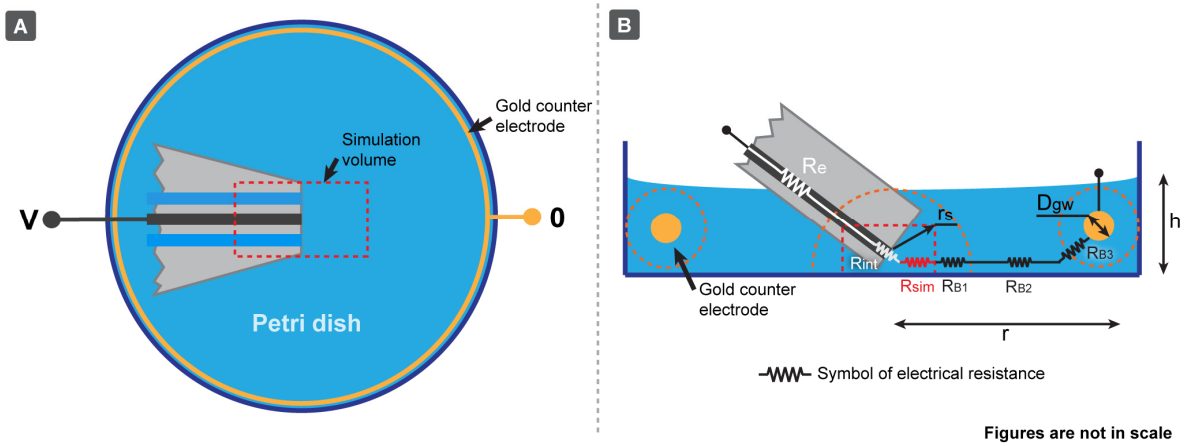

Figure S1. A schematic representation of the electrode setup; (A) plan view, (B) side view. The side view shows the series of resistances used in the simulations. The red dotted lines indicate the calculation regions; he central rectangle is was numerically simulated, the circular regions were analytically solved. The dish radius is denoted by "r", the liquid level is denoted by "h". All other parameter are defined in table S3 and S4.

Figure S2. Fluorescent and brightfield micrographs of pipette electoporated cells

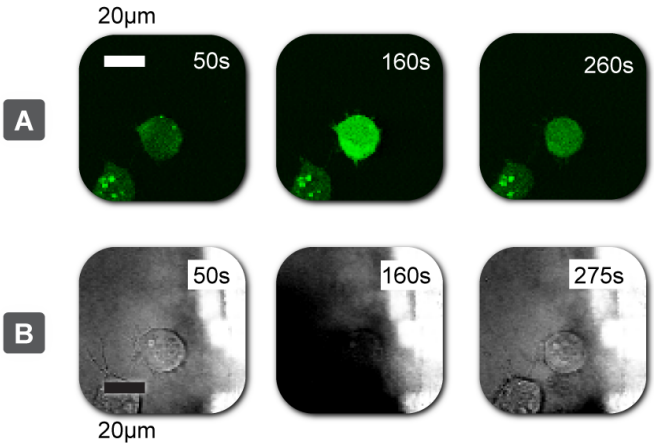

Figure S2. Fluorescent and brightfield micrographs at selected time points, for single cell electoporation using the pipetee encorporated electrode, for the graph in figure 3B . (A) Shows the calcium transport through imaging of the calcium green reporter dye. (B) displays the state of the cell prior and post electroporation as well as during calcium exposure. The lack of typan staining at the end of the experiment was a strong indication of membrane integrity at the end of the experiment.

Table S5. Characteristic parameters and operation conditions for the microfluidic superfusion pipette.

Description	Symbol	Value	Unit
Channel width	w	20	μm
Channel height	h	20	μm
Supply channel length	L_s	30	mm
Outlet channel length	L_o	10	mm
Electrode channel length	L_e	40	mm
Hydraulic conductance			
Supply channel	G_s	19	$nL/(s \cdot bar)$
Outlet channel	G_0	56	$nL/(s \cdot bar)$
Electrode resistance (*calculated*)	R_e	40	Ω
Operating settings			
Injection "on" pressure	P_{on}	0.15	Bar
Injection "off" pressure	P_{off}	0	Bar
Vacuum	V	-0.6	Bar
Inflow-Outflow ratio	I/O	4	
Outflow	Q_{out}	1.65	nL/s
Inflow	Q_{in}	6.6	nL/s
Approx. liquid exchange delay	τ	2.4	s