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► **To cite this version:**

Anne Calvel, Katia Grenier, David Dubuc, Marie-Pierre Rols. Real-time analysis of electroporation phenomenon of individual cells. *Electroporation-Based Technologies and Treatments*, Nov 2022, Ljubljana, Slovenia. hal-04281266

HAL Id: hal-04281266

<https://hal.science/hal-04281266>

Submitted on 12 Nov 2023

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Real-time analysis of electroporation phenomenon of individual cells

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INTRODUCTION

Electroporation (EP) is a promising approach for solid tumour treatment when combined with drug delivery, with high success rates (60-70% of complete remission) [1]. Leading single cell studies could provide a better understanding and mastery of EP at larger scales. Microdevices are adapted to perform such *in vitro* and *in situ* studies, while requiring low voltages.

MATERIALS AND METHODS

We perform *in situ* studies with a microdevice originally designed to analyse single cells by microwave dielectric spectroscopy [2]. It consists of three different parts: (a) a microfluidic channel (b) a metallic coplanar waveguide composed of a central signal conductor, between two ground planes, which contains a 10 μm gap in the middle and (c) a cell trap placed in the center of the microfluidic channel. The trap is located above the gap that constitutes the microwave sensing zone. The microfluidic channel, made of photoresist, is transparent, allowing to observe the trapped cell. Monitoring of cellular behaviour during EP using fluorescent biomarkers is also possible. A solution containing thousands of cells (PC3-GFP) is added to the fluidic inlet of the microdevice. Thanks to a continuous flow maintained by a syringe pump, the flow passes through the microfluidic channel. A cell can be captured by a mechanical trap specifically designed to catch and maintain a single cell during the study. Once a cell is trapped, electrical pulses are delivered in a buffer solution. A generator (AFG3022C Tektronix) applies pulses through tungsten electrodes positioned on both sides of the coplanar signal lines. We study the effect of different voltages (from 3V to 10V) in order to determine the permeabilization threshold. The impact of the pulses on the individual cell is visualized with two different fluorescent biomarkers: (a) calcein AM to assess membrane integrity and cell viability and (b) propidium iodide to quantify permeabilization. A real-time monitoring of the fluorophores' intensity is carried out.

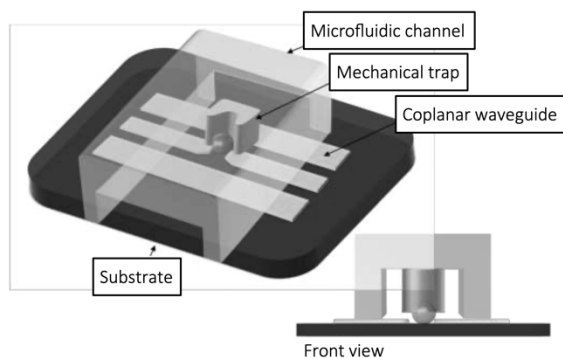


Figure 1: Schematics of the microdevice used for single cell EP and analysis [3].

RESULTS

8 pulses of 5 ms and 100 μs width at a frequency of 1 Hz are applied to different single cells. For pulses of 5 ms width, we observe that low electric field intensities (under 4.5 V) do not lead to permeabilization and maintain cell integrity. High field intensities (above 4.5 V) lead to membrane permeabilization as shown by the uptake of propidium iodide. These results are consistent with previous studies that have been realised for bulk EP [4].

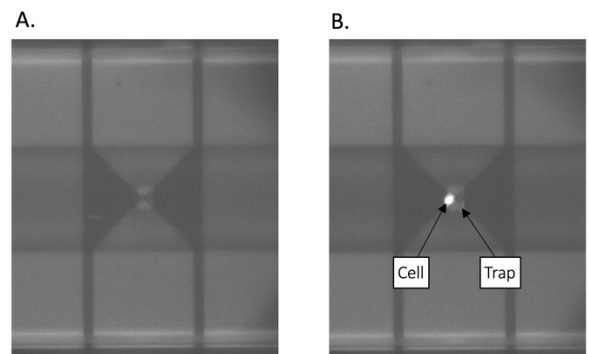


Figure 2: Image taken under fluorescence microscopy (m-cherry filter) of a single PC3-GFP cell trapped. A. After adding propidium iodide and before performing EP. B. After performing EP (8 pulses of 100 μs duration, 10 V magnitude, 1 Hz frequency).

CONCLUSIONS

We demonstrated that the microdevice previously developed enables *in situ* EP and real time analyses. The studies carried out allow to determine EP thresholds for different types of electrical pulses.

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