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Anne Calvel, Olivia Peytral-Rieu, Marie-Pierre Rols, David Dubuc, Katia Grenier. Extension to In Situ Single-Cell Electroporation of a Microwave Biosensor. *IEEE Microwave and Wireless Technology Letters*, 2024, 34 (6), pp.849 - 852. 10.1109/lmwt.2024.3391213 . hal-04651131

HAL Id: hal-04651131

<https://hal.science/hal-04651131>

Submitted on 17 Jul 2024

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Extension to *In Situ* Single Cell Electroporation of a Microwave Biosensor

Anne Calvel, *Student Member, IEEE*, Olivia Peytral-Rieu, *Member, IEEE*, Marie-Pierre Rols, David Dubuc, *Member, IEEE*, Katia Grenier, *Member, IEEE*

Abstract— This paper presents a biosensor firstly developed to perform microwave dielectric spectroscopy (MDS) analysis, which is evaluated for *in situ* electroporation (EP) at the single cell level. After demonstrating the possibility to characterize living cancer cells in the RF sensor by acquiring their dielectric properties, we then evaluate the use of this RF sensor to apply high electric field stimulus to trigger cell permeabilization. The impact of applied voltage and therefore electric field intensity on the number of permeabilized cells is defined, leading to the establishment of an EP threshold, which is EP conditions and cell dependent. This paves the way for further studies assessing the impact of *in situ* EP using the MDS method performed with a unique microdevice and consequent observation and understanding of the EP phenomenon.

Index Terms— microwave dielectric spectroscopy, electroporation, single cells, RF sensor.

I. INTRODUCTION

LEADING studies at the single cell level is crucial for a better understanding of cellular mechanisms and for studying cellular heterogeneity. Among the existing methods for studying cells, Microwave Dielectric Spectroscopy (MDS) is an attractive technique. It is a non-invasive and non-destructive method that allows cells to be studied directly in their culture medium, without the need for additional molecules such as biological markers [1], [2].

Among biological mechanisms of interest, electroporation (EP) is a promising method that raises new hopes in the field of oncology, especially with gene transfer and electrochemotherapy applications [3], [4]. Such methods are based on the application of pulsed electric fields that allow the entry of agents that are not or only slightly permeable, such as plasmids and cytotoxic agents. Although the efficacy and safety of these techniques have been demonstrated [5], fundamental research at the single cell level would help providing a better understanding and control of EP at larger

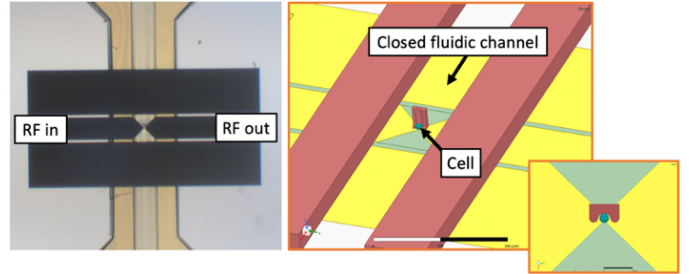


Fig. 1. Photography (left) and schematics (right) of the central part of the single cell microwave biosensor.

scales and could improve personalized medicine with the preliminary evaluation of EP conditions on patient cells.

Microdevices are suitable for carrying out such *in vitro* and *in situ* studies, while requiring only low electrical voltages while performing EP.

Different approaches have been developed in the past decades to perform on-chip single cell EP. Individualized static cells can be permeabilized by electrodes placed on both sides of a microfluidic channel [6]. A large amount of cells may also be electroporated on-chip, while located on the same dimensional plane [7], [8] and then individually analysed with optical techniques. Finally, single cells in suspension may also be permeabilized using a very low flow [9]. In this specific demonstration, both fluorescent images and impedance spectroscopy provide new insights on EP.

In addition to these analysing techniques, evaluating by MDS analysis the cellular response to *in situ* EP treatments would be of interest, as MDS has shown great promise for early detection of cells behaviour to different treatments [10], [11], [12]. Due to its frequency range, the microwave dielectric response also refers to intracellular modifications [13], which may not be observed optically as early as with the MDS technique. Therefore, co-integrating MDS analysis with optical fluorescence imaging in a unique biosensor could help improving our understanding in the EP phenomenon by providing real-time complementary information. The permeabilization state of the cell can be studied and compared by correlating the decrease in its capacitive and conductive contrasts with the entry of biomarkers into the cell. MDS analysis is expected to provide valuable information with the detection of subtle phenomena in the EP dynamics, the latter being not observable with the conventional approach of imaging.

For this purpose, we propose to perform *in situ* EP on single cells using a RF sensor originally designed to perform MDS analysis. After demonstrating the MDS capability of our sensor, we evaluate *in situ* EP with the previously designed

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This article was presented at the IEEE MTT-S International Microwave Symposium (IMS 2024), Washington, DC, USA, June 16-21, 2024.

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sensor. Different pulsed electric fields are then applied on living PC3-GFP cancer cells to define their impact with the monitoring of biomarkers to assess cell permeabilization.

We thus demonstrate the ability of the RF sensor to allow the application of pulsed electric fields that can be selected to trigger reversible or irreversible cell permeabilization. This paper is consequently presenting the biological materials and cellular solutions used during our experiments. We then present the miniature microwave biosensor developed to investigate the dielectric characteristics of individual cells measured in their culture medium.

II. BIOLOGICAL MATERIALS AND CELLULAR SOLUTIONS

A. Solutions and reagents

Gibco RPMI (GlutaMAX supplement, HEPES), geneticin (Gibco), pen-strep solution (100X) (Gibco), fetal bovin serum (FBS) (Hyclone), 0.05% Trypsin-EDTA (Hyclone) and Calcein AM are purchased from Thermo Fisher Scientific. DPBS with Calcium and Magnesium (1X) is purchased from Eurobio Scientific. Propidium iodide (PI) and the electropulsation buffer components (1 mM MgCl₂, 10 mM K₂HPO₄/KH₂PO₄, 250 mM of sucrose) are purchased from Sigma Aldrich.

B. Cell culture and preparation

Human prostate PC3-GFP (passages 10-30) cancer cells are cultured at 37°C under 5% CO₂ in RPMI medium supplemented with 10% of FBS, 1% geneticin and 1% pen-strep solution.

To be studied in the RF sensor, cells are suspended in RPMI medium and marked in Calcein AM at a final concentration of 1 mM.

III. MICROWAVE MICRODEVICE FOR SINGLE CELL STUDIES

The RF sensor that is used for EP studies is firstly evaluated to perform MDS analysis on single cells.

It includes a coplanar waveguide to vehiculate the electromagnetic wave, with a 5 μm capacitive gap on the central conductor, and a microfluidic channel with a cell trap, as presented in Fig.1, and inspired from [14].

The CPW accesses present a characteristic impedance in air of 50 Ω, which corresponds to (w,s) dimensions of (140,14) μm respectively. To focalize the electromagnetic field in the area of the cell, i.e at the location of the 5 μm large capacitive gap, the CPW central conductor is progressively narrowed from 140 μm to a width of 10 μm, from internal edge of the fluidic channel to the gap. A mechanical trap is placed at the center of a 50 μm-height microfluidic channel, which connects two fluidic inlets that allow the flow of a cellular suspension. The trap is designed to capture a single cell, as it is arranged perpendicular to the cellular flow within the channel and features a cavity for an individual cell to become lodged within. The trap is attached to the fluidic channel's lid. A spacing of 5 μm between the trap and the

bottom substrate is defined, ensuring the hydrodynamic cell's trapping and retention for both MDS and EP applications.

The microfluidic channel and trap, made of SU8 photoresist, are fabricated using photolithography above the coplanar waveguide. As the channel is transparent, it allows the observation of the cell trapping as well as fluorescent biomarkers monitoring to be observed once EP conditions are applied.

A. Demonstration of microwave dielectric spectroscopy on a single cell with the RF biosensor

Cells dielectric properties are obtained using a vector network analyzer and microwave probes. The measurements are preceded by a SOLT-type calibration. A cell solution (concentration of 10⁶ cells/mL) is introduced into the fluid inlet of the RF sensor. The flow rate is kept constant by a syringe pump connected to the microdevice's outlet. As soon as a single cell is blocked in the trap, the fluid inlet is continuously supplied with RPMI medium. The dielectric properties of the cell are obtained while considered with an electrical model composed of a capacitance in parallel to a conductance, and using a contrast method similar to existing methods [14].

The capacitive contrast of the cell of interest is obtained as follows: $\Delta C = C_{\text{cell}} - C_{\text{ref}}$, with C_{cell} the capacitance when the sensor is loaded with a cell contained in reference RPMI medium, and C_{ref} the capacitance when loaded by the reference RPMI medium alone. Similarly, the conductive contrast is obtained as follows: $\Delta G = G_{\text{cell}} - G_{\text{ref}}$, with G_{cell} the conductance when the sensor is loaded with a cell contained in reference RPMI medium, and C_{ref} the conductive part when the reference RPMI medium is only loaded.

The cells are studied over a frequency range of 40 MHz to 40 GHz. The S parameters are acquired using CaptureVNA software and the data processing used to extract the dielectric properties of interest is carried out using an internally developed Matlab program. The total duration of such a study is about twenty minutes, including cell trapping and release, as well as the acquisition of S parameters from the reference medium and the cell. The stability of the cell's electrical signature is also checked before application of EP. An example of the measured dielectric properties of a living PC3-GFP single cell is presented in Fig.2.

The capacitive and conductive contrasts between the cell and the reference RPMI medium are sufficiently large enough to allow the dielectric properties of the cell to be studied. Indeed, at 5 GHz, $\Delta C \approx -0.6$ fF and at 30 GHz, $\Delta G \approx -58.6$ μS. These obtained values are consistent with what can be found in the literature for MDS single cells studies [15], which validates the use of the RF sensor to provide dielectric characterization of single cells. Moreover, a previous study has demonstrated that the biosensor is adapted to perform real time monitoring of cells submitted to chemical treatments and the capacitive and conductive sensitivities have been estimated of at least 0.035 fF (at 5 GHz) [16] and 0.006 mS (at 40 GHz) respectively.

Next step of our investigation consists in assessing whether the RF sensor is suitable for applying *in situ* high energy pulsed electric fields to electroporate single cells.

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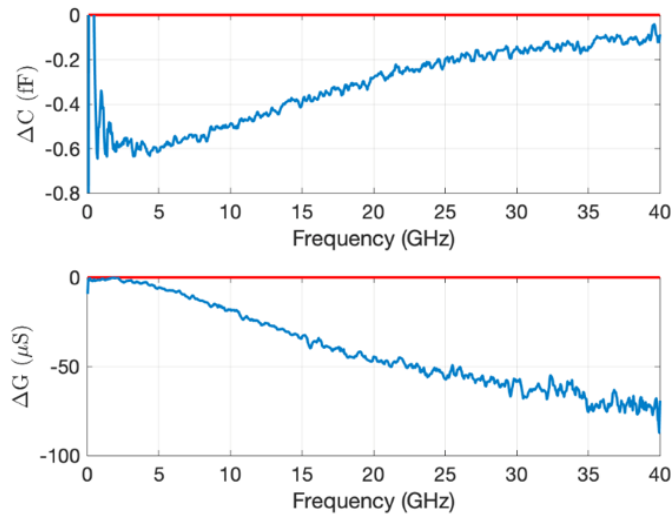


Fig. 2. Frequency spectra (40 MHz - 40 GHz) of a single PC3-GFP cell (in blue). Capacitive and conductive contrasts are obtained using RPMI culture medium as a reference (in red).

B. Demonstration of *in situ* single cell electroporation with the RF biosensor

To electroporate cells, electric pulses are applied by an electropulsator connected to tungsten electrodes placed on both sides of the coplanar waveguide of the microdevice. Electric pulses shapes are checked with an oscilloscope. The delivered pulses consist of a train of 8 pulses of 5 ms width at a frequency of 1 Hz of various amplitudes. The selection of these parameters is due to the fact that they are frequently used for *in vitro* and *in vivo* gene transfer applications [17]. The electric pulses are applied in electropulsation buffer containing PI at a final concentration of 100 μM . PI is indeed a non-permeant molecule used as a biomarker to assess cell permeabilization. Once cell membrane is damaged, PI binds to double stranded DNA and its quantum yield is enhanced 20-30 folds.

The effect of different voltages (from 0 V to 7 V) is carried out in order to determine the permeabilization threshold which is electrical field dependent. Our objective is to establish the minimum voltage required to permeabilize a significant proportion of cells.

Electrodes placement and fluorescent dyes monitoring are performed under a fluorescence microscope. A syringe pump is used to maintain a continuous flow within the fluidic channel. The test bench is presented in Fig.3.

The test protocol starts with the preparation of the cell solution, which is first marked with Calcein AM (1 mM). Calcein AM is a permeant molecule used as a biomarker to determine cell viability and thus allow the selection of living cells in the RF sensor. Once a single cell is trapped in the microdevice, the fluidic inlet of the RF sensor is supplied in electropulsation buffer with PI (100 μM). Electric pulses are then applied. To evaluate the impact of the applied pulses, PI uptake and Calcein AM leakage are visualized in real time under M-cherry and GFP filters, respectively, of a

fluorescence microscope. Fluorescence intensity for PI and Calcein AM is measured before and after EP by using ImageJ software.

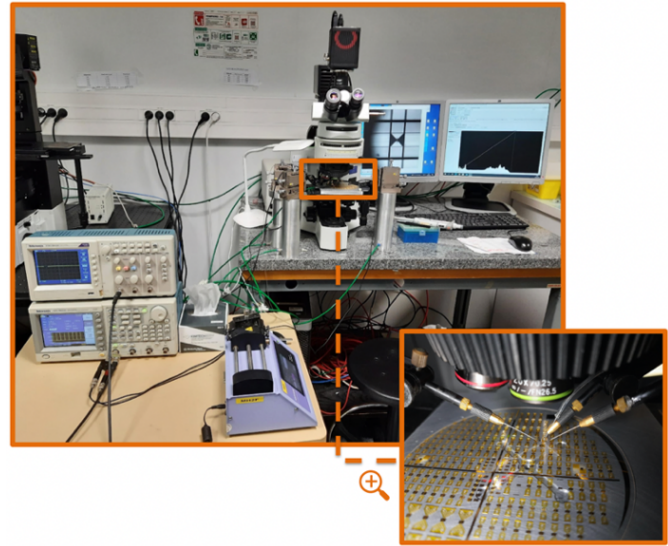


Fig. 3. Test bench for performing *in situ* EP, which is composed of an electropulsator, an oscilloscope, tungsten electrodes, a syringe pump, a fluorescence microscope.

EP of various amplitudes is applied on PC3-GFP single cells. An example of cell behaviour after the delivery of pulses exhibiting a 4.5 V amplitude is presented in Fig.4. We can observe the uptake of PI directly after the application of the pulses, as well as a decrease in fluorescence intensity of Calcein AM. These two observed phenomena confirm that the cell has been electroporated under these conditions.

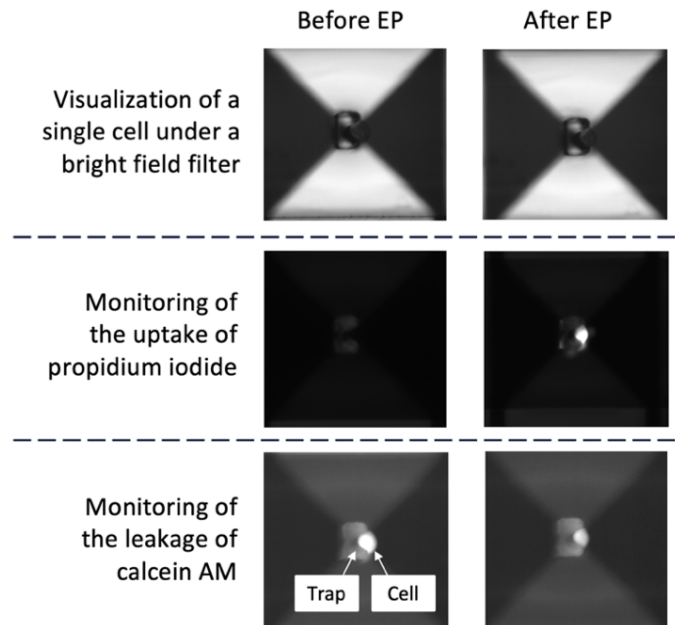


Fig. 4. Monitoring of *in situ* EP of a single PC3-GFP cell with fluorescent biomarkers. EP consists of a train of 8 pulses of 5 ms width at a frequency of 1 Hz and 4.5 V of amplitude.

As the electric field intensity is an essential parameter in

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the triggering of EP, the investigation on the impact of voltage (comprised between 0 V and 7 V) on permeabilization is conducted (see Fig.5).

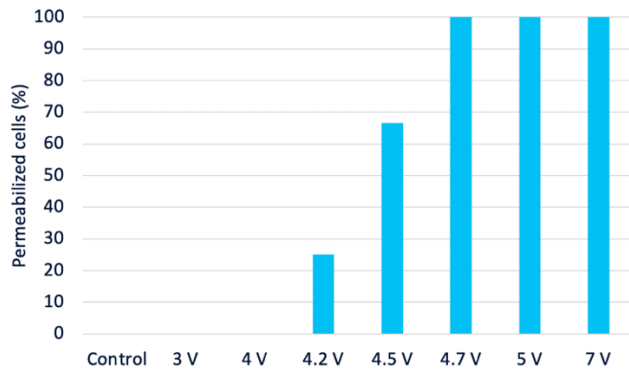


Fig. 5. Percentage of permeabilized cells following the application of 8 pulses 5 ms wide at a frequency of 1 Hz. For each voltage condition tested, at least 3 cells are studied.

We observe that voltages above 4.2 V need to be applied in order to trigger cell EP (i.e., the permeabilization threshold is found to be around 4.2 V). Such results are consistent with studies that evaluate the impact of EP at a macroscale with electric fields threshold of the order of few kV/cm [18].

Conducting extensive viability assays for the different voltage conditions would be of interest to determine whether the electroporation that occurs above 4.2 V is reversible or irreversible. Voltages beyond 4.7 V result in the permeabilization of all the cells under study. In this study, only the impact of the electric field intensity has been investigated. It could be of interest to conduct further studies regarding the number and width of pulses on the extent of permeabilization, aiming towards the best selection of electrical parameters for effective permeabilization while maintaining cell viability [19], [20].

The validation of the RF sensor for *in situ* EP enables MDS analysis of the EP phenomenon, as the acquisition of the cell's dielectric properties is possible before and after performing EP, due to the configuration of the mechanical cell trap, which enables the modification of the cell host medium during experiments.

IV. CONCLUSIONS

This paper targets to establish the possible use of a microwave biosensor adapted for single cell dielectric characterization for the application of *in situ* electrical stimuli.

First MDS analysis on a living PC3-GFP single cell is performed and demonstrated. With the same device, *in situ* EP is then performed. Cell behaviour with the use of fluorescent biomarkers is monitored. By studying the entry of PI into the cell and the leakage of Calcein AM, cell permeabilization is assessed. Once single cell EP is demonstrated in the RF biosensor, a further study is conducted to evaluate the impact of the voltage on cell permeabilization by applying amplitudes varying from 0 to 7 V. This allows us to determine a permeabilization threshold, which is specific to the employed

EP conditions and the cell line. This threshold corresponds to 4.2 V in this case.

This study therefore validates the possible use of a single cell and microwave-based biosensor for EP analysis. This paves the way for MDS studies of the impact of *in situ* EP of single cells, both stimulus and sensing parts being performed with the same microdevice. This configuration consequently opens perspectives toward further understanding of the EP phenomenon.

ACKNOWLEDGMENT

This work was partly supported by LAAS-CNRS micro and nanotechnologies platform member of the French RENATECH network.

The authors acknowledge the support of Université Fédérale Toulouse Midi-Pyrénées and Région Occitanie.

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