Toward the Real-Time Monitoring of Single-Cell Electroporation with Microwave Dielectric Spectroscopy

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***Abstract*—This project reports the research activities that were supported by the 2024 MTT fellowship program. It consisted of the development of an instrumentation able to perform the real-time monitoring of single cells subjected to electroporation treatments, based on Microwave Dielectric Spectroscopy (MDS). To evaluate such an approach, a conventional method based on real-time fluorescence microscopy is simultaneously used for characterizing electroporation, leading to a multiparametric set-up. Electroporation phenomenon has therefore been successfully observed on several cells. Obtained results show that MDS is adapted to monitor real-time intracellular changes in cells during electro-permeabilization.**

***Index Terms*—electroporation, microwave dielectric spectroscopy, real-time monitoring, RF sensor, single cells.**

I. INTRODUCTION

ELECTROPORATION (EP) is a method that transiently permeabilizes cell plasma membranes through the controlled application of pulsed electric fields. Recent advances in EP-based therapies are reshaping the treatment of cancer and genetic diseases [1] [2]. Such approaches consist in combining the local delivery of electric pulses with the administration of therapeutic molecules. Thereby, the effects of these molecules are potentiated, and treatments based on this synergy result in targeted and effective outcomes.

In order to address the challenges inherent in cell response variability, towards personalized medicine, it is essential to conduct studies at the single cell level. While conventional characterization methods rely on optical ones, the development of complementary approaches capable of monitoring intracellular mechanisms would improve our understanding of EP phenomenon and its short- and long-term dynamics. Microwave Dielectric Spectroscopy (MDS) is a promising approach, as it allows access to the intracellular content of the cells, that can be directly studied in their culture medium in a non-invasive, non-destructive, non-ionizing and rapid manner [3].

This project therefore consisted in enabling the real-time monitoring of single cells subjected to *in situ* EP treatment, using a dual-complementary approach that combines MDS and a conventional optical technique in order to correlate the two. A complete instrumentation integrating both MDS and fluorescent microscopy was first established and then used to evaluate in real time the EP phenomenon on individual cells.

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II. PROJECT OUTCOMES

A. Achievement of a complete MDS and optical instrumentation

We study THP-1 cells, derived from a human leukemia monocytic cell line. Single cells are mechanically isolated in a microdevice, which integrates a coplanar waveguide and a fluidic microchannel, which design is detailed in [4]. Previous studies allowed us to validate the use of such devices for 1. MDS characterization and 2. *in situ* EP with fluorescence microscopy monitoring [5].

We developed a new instrumentation, as shown in Fig. 1, that allows to co-integrate:

- A microfluidic system for single-cell trapping.
- *In situ* EP.
- Real-time MDS monitoring.
- Real-time fluorescence microscopy monitoring.

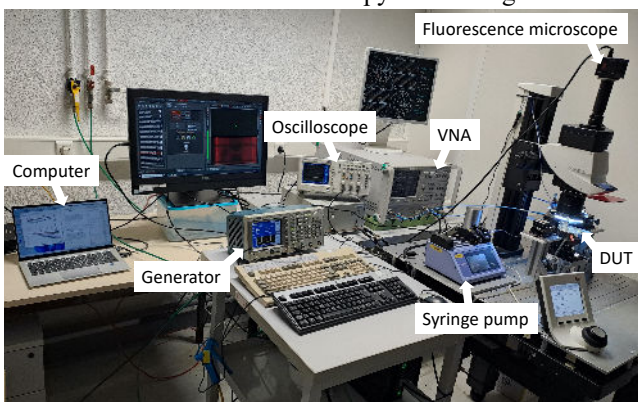

Fig. 1. Instrumentation for simultaneous MDS and fluorescence microscopy monitoring of *in situ* single-cell EP.

Both stimulus and sensing are performed through RF probes, placed on both sides of the coplanar waveguide of the microdevice. MDS measurements are performed at a low power of -7 dBm, over a frequency range from 40 MHz to 40 GHz. The dielectric properties of the single cells are studied using a differential approach, in terms of capacitive and conductive contrasts, ΔC and ΔG, extracted from S parameters [4]. To trigger EP, we select electrical pulse parameters (e.g., pulse number, pulse width, frequency) similar to those used in clinical practice [6]. Different pulses voltages have been

evaluated, based on previous voltage-conditions studies.

Optical monitoring of EP is achieved by assessing the fluorescence emission of two biomarkers in the cell: Propidium Iodide (PI) and calcein AM. Prior to trapping, cells are labeled with calcein, which is a permeant molecule indicative of cell viability. PI is introduced just before pulse delivery, and allows to label cells with compromised membranes as it is a non-permeant molecule. PI uptake and calcein AM leakage reflect cell permeabilization state.

B. Functionalities validation and use for EP phenomenon monitoring

The different functionalities of the developed instrumentation are evaluated. Fig 2 illustrates an example of simultaneous MDS analysis and fluorescence microscopy characterization performed on the same single cell subjected to irreversible EP treatment.

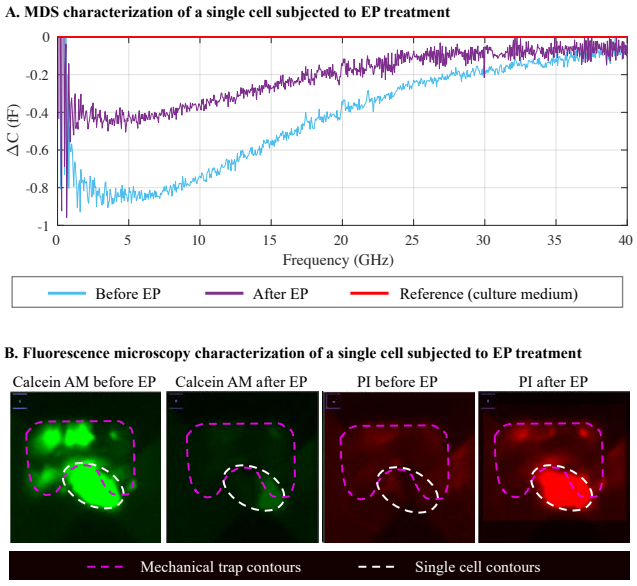

Fig. 2. Simultaneous co-characterization of single cell EP with A. MDS and B. fluorescence microscopy.

Panel A of Fig. 2 shows the frequency spectrum of capacitive contrast of a single cell before (in blue) and after electrical pulses delivery (in purple). The two states can be distinguished at frequencies below 35 GHz. Before pulse delivery, the level of contrast is representative of a living cell [7]. After pulse delivery, the absolute value of ΔC decreases of about 52%, at the frequency which exhibits the maximum contrast, e.g. 5 GHz. Such a decrease in absolute value is consistent with the presence of exchanges between the intra- and extracellular media [8]. After the application of the pulse train, the dielectric contrast levels are similar to those corresponding to a cell that is severely affected, or even dead [7]. Panel B of Fig. 2 illustrates the evolution of the calcein AM and PI intensities before and after the train pulses. Cell contours are shown in white dotted lines and the mechanical trap contours are shown in pink dotted lines. The initial levels of fluorescence are coherent with a living state. The decrease in calcein AM intensity after pulses delivery reflects the leakage of calcein AM, indicative of electro-permeabilization. The increase in intensity of PI reflects the uptake of PI, also indicative of a permeabilized cellular state. This trend aligns with the MDS measurements, emphasizing that the cell experiences significant internal and membrane changes.

The studies validate the use of the developed instrumentation to perform *in situ* EP on single cells, with simultaneous MDS and fluorescence microscopy characterization. Real-time monitoring of the EP phenomenon has also been performed on several single cells. The results demonstrated good correlations between MDS and fluorescence microscopy characterization, as well as consistent repeatability. MDS offers access to real-time intracellular variations, providing valuable insights into the mechanisms of the EP phenomenon and cell state.

III. Fellowship Impact and Career Plans

I am deeply honored and grateful to the IEEE MTT-S for awarding me the MTT-S Graduate Fellowship for Medical Applications in 2024. This prestigious recognition has provided a significant source of motivation for my research activities, both for my PhD and for my future career.

The fellowship allowed me to attend the 2024 IMS, which was a valuable opportunity to interact with the participants, including renowned scientists, and acquire knowledge in the latest advancements in the various microwave applications.

For my future career, I intend to continue contributing in biophysics research, and I am delighted to start a postdoctoral position.

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