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Microwave Monitoring of Single Cell Monocytes Subjected to Electroporation

Amar Tamra, Associate Member, IEEE, David Dubuc, Member, IEEE, Marie-Pierre Rols, and Katia Grenier, Member, IEEE

Abstract—This paper presents the monitoring of single cells subjected to electroporation using microwave dielectric spectroscopy. The experimental results showed first a clear distinction between two cell states: viable cells and affected ones by a chemical treatment (Saponin). It also pointed out a high correlation ($R^2 > 0.94$) with biological standard techniques in detecting the two types of electroporation: the reversible and irreversible ones. The developed microfluidic and microwave-based sensor exposes a decrease in the capacitive and conductive contrasts of the investigated single cells treated by irreversible electroporation indicating damages at the cellular level, while cells under reversible electroporation present a similar dielectric response to that of the nontreated cells. This result corresponds to results frequently employed in biological studies. More interestingly, a study of the kinetics of the cell’s damage induction over time, by electroporation, has been experimentally done, which makes microwave dielectric spectroscopy an attractive technique for cell’s electroporation researches.

Index Terms— Biosensor, dielectric spectroscopy, electroporation, microwave analysis, single cell.

I. INTRODUCTION

CELL membranes are phospholipidic bilayers, that constitute barriers between the intracellular and the extracellular domains. Accessing the intracellular compartments of cells, using electroporation for instance, has shown a strong interest for various biomedical applications. Electroporation notably enables the internalization of foreign molecules into the cell, elimination of microorganisms, insertion of proteins into cell membrane, and cells fusion [1]. The development of a physical method, ElectroPoration EP (or ElectroPermeabilization), that consists in using the application of external well controlled electric field pulses [2], enabled to transiently or permanently permeabilize the plasma membrane. This process allows the entry or exit of specific molecules of interest, such as small molecules (drugs in Electrochemotherapy) and macromolecules (genetic material in Electrogenotherapy) into the cell cytoplasm [3]. Electroporation depends on a number of electric pulse parameters, such as intensity, number, duration, and repetition frequency of the pulses [4]–[6]. Electric field intensity is the deciding parameter inducing permeabilization. Rols and Teissié [7] reported that an increase in the number of pulses enhances the rate of permeabilization and increasing the duration of the pulses is crucial for the penetration of macromolecules compared with small molecules. Puchiar et al. [8] showed that the uptake of exogenous molecules into cells in vitro stays at similar levels even at frequencies up to 8.3 kHz compared with the classical used frequency of 1 Hz. After all, the choice of the electrical parameters to be used depends on the type of application, the treated cells or organs and the type of molecules used.

The first studies on electroporation reported cell death caused by the irreversible permeabilization (IrReversible Electroporation-IRE) of cell membranes. This approach is nowadays used in tumor ablation. It consists in applying to the cell short pulses at high voltage, causing the formation of permanent nanometric defects in the cell membrane. This permanent permeability leads to changes in cellular homeostasis and cell death [9]. Unlike the IRE, reversible permeabilization of the plasma membrane can be induced by applying external electrical impulses with well-chosen amplitude and duration (few kV/cm and few microseconds), to facilitate the passage of little or nonpermeant molecules, without affecting cell viability [7]. Once the electric pulses are submitted to the cell, its membrane’s electric potential $\Psi$ is modified. The permeabilization occurs when the sum of the native transmembrane potential $\Delta \Psi_o$ and the induced transmembrane potential $\Delta \Psi_i$ exceeds a threshold value ($\approx 200$ mV) [10].

Despite progress in the field of electroporation and electrochemotherapy, enlarging our knowledge about the associated mechanisms and kinetics remains of high interest. The routine methods used for the evaluation of the effect of electroporation on the cells permeability and viability are indeed effective. However, they require the large amount of time as well as the large volumes of biomaterials, but above all are abusive for the cells. For example, when using classical microscopy combined to the use of fluorescent markers (calcein AM ($AM = \text{acetoxymethyl}$), Propidium Iodide (PI), and so on.), the cells can no longer be used for additional tests. The same problem applies when using biochemical tests, such as Tetrazolium Reduction Assay MTT (3-(4, 5-dimethylthiazolyl-2)-2', 5-diphenyltetrazolium bromide), that reflects the cell’s mitochondrial activity.

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In addition to these techniques, the biological research domain is witnessing the development and the employment of new physical methods, such as atomic force microscopy, electronic microscopy, and electrical spectroscopy. The study of the electrical properties of biological materials has shown indeed a wide interest [11], [12]. At low frequencies, up to few hundreds of MHz, the membrane’s state can be revealed [13], [14]. Nevertheless, the efficacy of the technique of electrical impedance spectroscopy is limited at the cell membrane level. Whereas by applying high frequency electromagnetic waves (beyond hundreds of MHz), the waves can overcome the cell membrane and interact directly with the intracellular compartments [15], [16]. In addition, the demonstration of microwave sensing at the single cell level has already been performed [17]–[21]. It enables the evaluation of cells suspension heterogeneity, which is of high interest in bio-physical processes investigation and understanding.

This paper reports our work on a microwave and microfluidic-based biosensor, able to perform dielectric spectroscopy of the single cell and to monitor the intracellular effects of both reversible and irreversible electroporations. Compared with [22], reversible electroporation sensing with microwave dielectric spectroscopy is demonstrated. Supplementary information on protocol, dielectric threshold determination and electroporation results are also given.

This paper is organized as follows. Section II shows the microdevice architecture combining microwave and microfluidic functionalities, as well as the experimental protocol used. Section III describes the choice of the electrical parameters used for electroporation. Microwave modeling of a single cell, the calibration procedure and the electrical response of a single cell to a chemical treatment (saponin) are presented in Section IV. Sections V and VI will show, respectively, the efficacy of the dielectric spectroscopy technique in reflecting cell state after reversible EP and IRE, and the experimental microwave monitoring of the kinetics of the cells response to irreversible electroporation. The solid correlation between the microwave readout and the results obtained with biological methods sharply reflects the efficacy of the microwave dielectric spectroscopy technique to reveal the kinetic effects of cell’s electroporation. Finally Section VII summarizes and concludes this paper.

II. MICROWAVE BIOSENSOR AND EXPERIMENTAL PROTOCOL

A. Microwave Microfluidic Biochip

This paper is based on a microwave and liquid-based sensor developed by Chen et al. [17], that presents a microfluidic channel allowing the process of biological materials (pure culture medium or medium with cytotoxic agents or suspension of cells). This microwave sensor is moreover markerless, contact-less, and permits to analyze the single cell within its culture medium. The microdevice, shown in Fig. 1, consists of a coplanar waveguide with a capacitive gap in the center. A microfluidic channel is placed on top of the microwave circuit and a mechanical trap is located over the capacitive gap, which captures one single cell. When the electromagnetic waves interact with the biological sample, a reflection of the cell’s dielectric properties is obtained, which makes it possible to reveal, in real time, the state of the cell. The mechanical trap configuration not only permits to immobilize a single cell in the sensing area, where electromagnetic waves are concentrated, but it also enables to easily use all kinds of surrounding liquid to the cells (including ionic liquids and not only sucrose, conversely to the dielectrophoretic solution [18]–[21], and to add chemical treatment to the culture medium of cells if required.

B. Experimental Protocol

Human monocytes THP1 cells are used in our paper. They are cultured in culture flasks with the Gibco Roswell Park Memorial Institute 1640 medium premixed with 10% of fetal bovine serum and a mixture of antibiotics (100 Unity/mL of penicilllin and 100 μg/mL of streptomycin). Cells are incubated at 37 °C and under 5% of CO2 for proliferation.

For electroporation, electric pulses are delivered to the cells using a Betatech pulse generator with two stainless steel electrodes with a distance of 4 mm. Cells are put in a pulsation buffer ZAP prior to pulsation, which has the following composition: 10 mm K2HPO4/KH2PO4, 1 mm MgCl2, 250 mm of sucrose, pH 7.4. The used electrical parameters are defined based on the type of electroporation, we wanted to induce: a reversible or irreversible one. The definition of these parameters is explained in the Section III.

The protocol used is shown in Fig. 2. First, cells are electrically stressed, then reincubated at 37 °C and 5% CO2 for 3, 5, 7 or 24 h. After this incubation time, cells are harvested and suspended in RPMI medium. The protocol used is shown in Fig. 2. First, cells are electrically stressed, then reincubated at 37 °C and 5% CO2 for 3, 5, 7 or 24 h. After this incubation time, cells are harvested and suspended in RPMI medium.
in order to compare both results. The microwave test setup is shown in Fig. 3. The cell suspension is injected with the syringe pump in the microfluidic channel of the device under test, which is connected to the network analyzer via cables and microprobes. The whole setup is monitored by a CCD camera. For each condition, at least ten microwave measurements have been realized.

In order to evaluate the permeabilization rate after application of electric pulses, cells are put in the presence of PI. It is a small nonpermeant molecule, that intercalates in the nucleic acids (DNA or RNA) of the nucleus of cells that lost their membrane integrity [23]. We visualize the fluorescence emitted with the DMIRB-cool snap FxHQ2 (mCherry Filter) and the quantification of the permeability rate is assessed with the flow cytometer (Facscalibur, IPBS).

In this paper, the viability of our cells after any kind of treatment is estimated either with the flow cytometer coupled to the PI molecule or with the Trypan Blue dye. The latter is a specific dye that instantly penetrates into the cells with permeabilized/damaged cytoplasmic membranes.

III. CHOICE OF ELECTRICAL PARAMETERS FOR ELECTROPORATION

Not all types of cell lines respond in the same way, when subjected to electric pulses. For this reason, a mandatory step was applied: the study of THP1 response to electric pulses in term of permeability and viability in order to choose the convenient intensity of the electrical pulse throughout this work. The other parameters (number, duration, and frequency of the pulses) are kept constant.

After treating different groups of cells with different intensities of electric field (without EP for the control test and from 0.4 to 2 kV/cm for the other groups), we quantified the percentage of permeabilized cells (Fig. 4). The photos show (a) THP1 cells in white light and (b) the fluorescence emitted from nontreated (negative control) and (c) permeabilized cells (at 1 kV/cm). The fluorescing cells present in the negative control photo represent the basal level of cell death of this cell line. Fig. 4(d) shows the dependence of membrane permeabilization on the electric field intensity. The penetration of PI is significantly detected as of 0.6 kV/cm and reaches its maximal value around 1 kV/cm.

A good compromise should be done when choosing the right parameters in a way that we have a high permeabilization rate along with preserving a high viability rate. For this purpose, viability assays are conducted 24 h after electroporation. Fig. 5 shows the percentage of viable cells 24 h after electroporation with two biological methods: the flow cytometry and the Trypan Blue test. As expected, when increasing the amplitude of the electric pulses and the cell viability decreases. This is due to the inability of the cells to reseal the pores and recover after this shock. Therefore, for the rest of the study, we use the following parameters, based on the desired type of electroporation:

1) reversible electroporation: 8 pulses of 1 kV/cm with a pulse duration of 100 μs and at a frequency of 1 Hz;
2) irreversible electroporation: 8 pulses of 1.5 kV/cm with a pulse duration of 100 μs and at a frequency of 1 Hz.

IV. MICROWAVE MODELING OF SINGLE CELL

The microwave measurements are performed on wafer in a range of frequencies from 40 MHz to 40 GHz. The biosensor, shown in Fig. 1 and described in [24], is associated to an electrical model, which consider the coplanar waveguide access lines, the SU8 microfluidic walls and the capacitive sensing zone as shown in the Fig. 6(a).
Prior to any measurement, we characterize two calibration elements: 1) the thru with the SU8 walls, which corresponds to the biosensor structure where the sensing zone is suppressed and replaced by a thru connection and 2) the thru without the SU8 walls, for which the SU8 layer is suppressed. Fig. 6(b) shows these calibration elements and their associated electrical schematics. With these elements and by fitting the S parameters of the measurements with those of the models, we determine the characteristic impedance ($Z_0$) and attenuation coefficient (α) of the access lines as well as the SU8 walls’ capacitance. Finally, using ABCD matrix de-embedding technique, we extract the sensing impedance of the sensing zone from the measured S-parameters of the biosensor [25], [26].

Then we follow the following procedure: the culture medium (without cells) is first injected and measured; this is our reference medium. After the injection of the cell suspension, a second set of measurements is performed once a single cell is captured inside the blocker, on top of the sensing zone. The extracted information from the sensing volume [17], the effective capacitance $C_{\text{eff}}$ and conductance $G_{\text{eff}}$, are compared with the reference medium by calculating the respective contrasts

$$\Delta C_{\text{eff}} = C_{\text{cell}} - C_{\text{reference medium}}$$  \hspace{1cm} (1)
$$\Delta G_{\text{eff}} = G_{\text{cell}} - G_{\text{reference medium}}$$  \hspace{1cm} (2)

where $C_{\text{cell}}$ and $C_{\text{reference medium}}$ correspond to the respective capacitance when the sensor is loaded with a cell or with the reference medium only, and similarly for $G_{\text{cell}}$ and $G_{\text{reference medium}}$ that represent the conductance in the two previously mentioned configurations.

The plain curves in Fig. 7 represent the respective measured spectra of (a) capacitive and (b) conductive contrasts of both a living and a saponin-treated cell (0.02%). Saponin is a detergent, that makes the lipid bilayer permeable to macromolecules. The saponin-lysed cells do not reseal, indicating thus an irreversible damage of the lipid bilayer [27].

As shown in Fig. 7, both contrasts decrease (in absolute values) after exposure to the saponin molecule for 15 min, reflecting the permeabilization of the cell membrane. Each condition was tested at least three times ($n = 3$), and a total of 50 cells is analyzed per condition. This result is in agreement with other publications. In particular, the work presented in [28] shows distinct differences in return loss $S_{11}$ and insertion loss $S_{21}$ of living and dead biological cells.

These contrasts variations ($\Delta C_{\text{eff}}$ and $\Delta G_{\text{eff}}$) clearly point out the dispersive behavior of the high water content of the cell. To model this phenomenon, we consider a physical electrical schematic, derived from the Debye model [29] (dashed curves).

The physical (Debye-based) model of the single cell consists in a capacitor ($\Delta C_{\text{cell}}$) placed in series with a resistor ($\Delta R_{\text{cell}}$), which reflects the relaxation phenomenon of the aqueous solution inside the cell. The relationships between the effective capacitive and conductive contrasts and the Debye-based model are

$$\Delta C_{\text{eff}} = \frac{\Delta C_{\text{cell}}}{1 + (\Delta C_{\text{cell}} \times \Delta R_{\text{cell}} \times \omega)^2} = \frac{\Delta C_{\text{cell}}}{1 + \left(\frac{f}{f_r}\right)^2}$$  \hspace{1cm} (3)
$$\Delta G_{\text{eff}} = \frac{(\Delta C_{\text{cell}})^2 \times \Delta R_{\text{cell}} \times \omega^2}{1 + (\Delta C_{\text{cell}} \times \Delta R_{\text{cell}} \times \omega)^2} = \frac{\Delta C_{\text{cell}} \times \omega^2}{1 + \left(\frac{f}{f_r}\right)^2} \times \frac{f}{f_r}$$  \hspace{1cm} (4)
TABLE I

<table>
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<tr>
<th></th>
<th>$\Delta C_{\text{cell}}$</th>
<th>$f_\tau$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Living cell</td>
<td>-0.65 fF</td>
<td>19 GHz</td>
</tr>
<tr>
<td>Affected cell</td>
<td>-0.30 fF</td>
<td>19 GHz</td>
</tr>
<tr>
<td>Threshold</td>
<td>-0.47 fF</td>
<td>-</td>
</tr>
</tbody>
</table>

where $f_\tau$ corresponds to the relaxation frequency of the intracellular aqueous cytoplasm and is given by

$$f_\tau = \frac{1}{2\pi \times \Delta C_{\text{cell}} \times \Delta R_{\text{cell}}}.$$  \hspace{1cm} (5)

With this model, $\Delta C_{\text{cell}}$ and $f_\tau$ for both living and affected cells have been extracted. These values are summarized in Table I. First, the $f_\tau$ value is not changed when the cell is affected compared with the living cell. Conversely, $\Delta C_{\text{cell}}$ values exhibit significant variation ($-53\%$) between living ($-0.64$ fF) and affected ($-0.30$ fF) cells.

This may be explained by the fact that the affected cell features a permeable membrane exhibiting greater molecular and ionic exchanges with the surrounding medium. The capacitive contrast is consequently less important when liquid exchanges arise, to reach equilibrium between the intra and extracellular media. This study allowed us to define a threshold value between alive and dead (i.e., affected) cells at the middle of the measured dynamics: $\Delta C_{\text{threshold}} = -0.47$ fF. This threshold will be used in the next paragraph for cell screening depending on the viability.

V. EVALUATION OF ELECTROPORATION INTENSITY EFFECT

As explained earlier, the reversibility of the electroporation depends on the electrical parameters applied: a low voltage will induce a reversible electroporation and the cell will return to its normal state, whereas a high voltage will induce permanent damages and the cell will not be able to recover.

First, we have evaluated the ability of the dielectric spectroscopy to detect the effect of using different electric field intensities on living cells. For that, cells are subjected to 1 or 1.5 kV/cm (reversible and irreversible EP respectively) before being incubated for 24 h at 37 °C. At least ten cells are measured in each condition. Next, a step of results sorting and classification is done, by referring to the thresholds previously established.

The mean values of the capacitive contrasts, are presented in Fig. 8. The percentages of cells, viable or affected by the electroporation, are also shown. In comparison to the control cells (nontreated), that present 88% of viability rate, reversible electroporation does not induce an important effect on the cells viability ($\sim95\%$); whereas by applying IRE, a decrease in the viability level down to 55% is observed.

Trypan blue test (biological test) is used to further validate the efficacy of our technique (cf. Fig. 9). A statistical analysis revealed a sharp correlation between the two methods of analysis ($R^2$ “$\Delta C_{\text{cell}}$/Biological method” = 0.98) indicating the robustness of the dielectric spectroscopy technique in detecting the effect of the reversible and irreversible electroporation on THP1 cells.

VI. MONITORING OF THE KINETICS OF IRREVERSIBLE ELECTROPORATION

After validating the success of the dielectric spectroscopy in detecting the effect of reversible and irreversible EP on cells, we have investigated the kinetics related to cell’s response to irreversible electroporation.

Fig. 10 shows the percentage of viable cells after electroporation with both biological and microwave techniques (only the capacitive contrast of the cells). In comparison to the control cells presenting a viability rate of 73%, IRE induces a viability decrease down to 50% after 3 and 5 h and down to 40% after 7 h post IRE. 24 h after electroporation, the viability rate increases up to 50% and we hypothesis that it is due to the proliferation of remaining viable cells after treatment, as the cell cycle of THP1 is of the order of 24 h. After performing a statistical analysis, a robust correlation coefficient $R^2$ between the microwave measurements compared with flow cytometry
It also enables the monitoring of these damages over time. This article has been accepted for inclusion in a future issue of this journal. Content is final as presented, with the exception of pagination.

**Fig. 10.** Monitoring of kinetics of the response of THP1 after exposure to irreversible electroporation and correlation between microwave measurements and biological method.

Data \( R^2 = 0.96 \) for correlation between \( \Delta C_{\text{cell}} \) and the biological method is deduced. These correlations audibly show the ability and robustness of our microwave and microfluidic-based technique in giving access and monitoring the effects of electroporation on cells. Furthermore, the microwave technique presents a number of assets making it very promising in the field of research, namely the fact that it is nondestructive, marker less, and compatible with cells directly measured in their culture medium.

**VII. CONCLUSION**

In this paper, we demonstrated that the microwave dielectric spectroscopy performed at the level of a single biological cell is able to discriminate the reversible and irreversible electroporations. All the more, it does not only reveal the cell’s damages after treatment with irreversible electroporation but it also enables the monitoring of these damages over time. Aside from dielectric measurements, biological investigations (based on flow cytometry and trypan blue staining) undeniably indicate that microwave results have a strong correlation \( R^2 \) of 0.96 on \( \Delta C_{\text{cell}} \) with these biological standard techniques. These results confirm that the proposed technique may contribute to having more insights and information the effect of electroporation and electrochemotherapy on different types of cells. It may therefore enrich the technique’s arsenal of single cell analysis, within the context of the fight against cancer and beyond.

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