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BIOIMPEDANCE SPECTROSCOPY FOR NON-INVASIVE CHARACTERIZATION OF CELLULAR CHANGES

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Abstract:
This study investigates whether bioimpedance spectroscopy (BIS) is a suitable method for non-invasive characterization of fundamental biological changes in cells. Experiments were designed to model changes in the extracellular and intracellular spaces and to study the effect of cell proliferation on impedance data. BIS measurements were carried out using a self-developed BIS measuring system using graphene-based electrodes. Impedance spectra were evaluated using the Cole–Cole model, allowing the identification of key impedance parameters and their correlation with biological changes. The results indicate that intracellular changes induces significant changes in high-frequency resistance (R_∞) and capacitance (C), while cell proliferation are primarily reflected in the α relaxation parameter. Changes in the extracellular environment do not result significant differences in the model parameters, which confirms the method's selectivity for intracellular processes. The results reinforce that BIS could be a suitable tool for label-free, non-invasive monitoring of cell status, with significant potential applications in biomedical diagnostics.

Keywords: *Bioimpedance spectroscopy; Cole–Cole model; Cellular analysis, In vitro diagnostics.*

Introduction

Study of biological systems is essential for understanding how living organisms function and for developing diagnostic methods. Non-invasive analysis of biological samples allows real-time monitoring of the state of cells and tissues without compromising their integrity. Among non-invasive approaches, electrical impedance spectroscopy (EIS) known as bioimpedance spectroscopy (BIS) when applied to biological samples is considered a remarkable method due to its simplicity, cost-effectiveness, and high sensitivity [1]. BIS is widely used as a non-invasive method to study the condition of cells and tissues [1-4], as well as in medical diagnostics, including cancer research [5,6].

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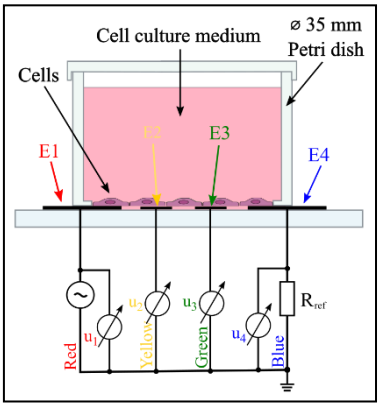
Changes in cell viability and biological characteristics can be characterized by measuring their electrical properties, as the impedance spectrum is significantly influenced by the electrical properties of the cell membrane and the intracellular and extracellular spaces. BIS spectra are evaluated using equivalent circuit models, most commonly the Cole–Cole model. Using the Cole–Cole model, the biological sample can be characterized by five direct parameters (R_ω, R_0, $R_0 - R_\infty$, δ, τ), which can be correlated with biological components [7]. However, the large number parameters complicate the direct physiological interpretation of the circuit output, particularly in complex biological systems [7].

The authors have introduced a self-developed four-electrode BIS system and the corresponding measurement plate, which have previously been validated in live-cell experiments [8,9]. The authors aim to correlate parameters derived from BIS measurements with physiological processes and fundamental cellular biological characteristics. These findings may contribute to the interpretation of in vitro and human clinical BIS results, thereby facilitating the early detection of diseases and the development of personalized therapies.

1. The developed BIS measurement technique

BIS measurements are carried out using a self-developed, modified four-electrode method introduced by Vizvari et al [9]. This technique applies common-mode rejection to reduce measurements errors caused by the excitation electrodes and residual impedances. The principle of common-mode rejection is based on the signal generator being connected directly to one electrode, while the ground is connected to the other electrode through a resistor, resulting in a constant offset in potential levels. The digital compensation and division perfomed during impedance calculation eliminate errors that occur in the symmetrical measurement circuit. Picture 1 illustrates the operating principle of the self-developed in vitro BIS technique.

In the configuration shown in Picture 1, the series resistor (R_{ref}) creates a constant voltage shift in the measuring cell, causing excitation electrodes (E1 and E4) to operate at a non-zero potential, developing an electrical double layer on the both of the electrodes. Current flows between the voltage generator (E1) and electrode E4. The measuring electrodes (E2, E3) are positioned separately to minimize the effect of the double layer.



Picture 1. *Schematic drawings of the self-developed in vitro BIS technique application in case of the self-produced graphene plates [9]*

The spectrum of the measured sample (Z) is calculated at each frequency point using the potential values u_2, u_3 and u_4 according the following formula [9]:

$$Z = R_{ref} \frac{u_2 - u_3}{u_4} \quad (1)$$

The most commonly used model-based approach for evaluating measured spectra is the Cole–Cole model, which describes the frequency-dependent behavior of biological systems using an equivalent circuit. The equivalent circuit equation describing the Cole–Cole model is given by the following form [9]:

$$Z(j\omega) = R_\infty \frac{R_0 - R_\infty}{1 + (j\omega\tau)^\alpha} \quad (2)$$

where $Z(j\omega)$ is the complex impedance, R_∞ is the resistance related to the ∞ frequency, R_0 is the resistance related to 0 Hz frequency, τ is the time constant, α is the exponent parameter ($0 < \alpha \leq 1$), ω is the angular frequency and $j=\sqrt{(-1)}$.

2. Experiments and Results

The cellular biological characteristics associated with the BIS parameters were validated through a series of in vitro experiments. In each experiment, only a single component (e.g., the extracellular space) was selectively modified to isolate the effects. This approach made it possible to determine which BIS parameters are affected by changes in each component.

For the experiments, a self-developed BIS measuring device and compatible, custom-made plates with graphene electrodes were used (Picture 2).



(a) **(b)**

Picture 2. *BIS experimental setup: (a) assembled BIS measuring system; (b) connected costum-made graphene BIS measuring plates*

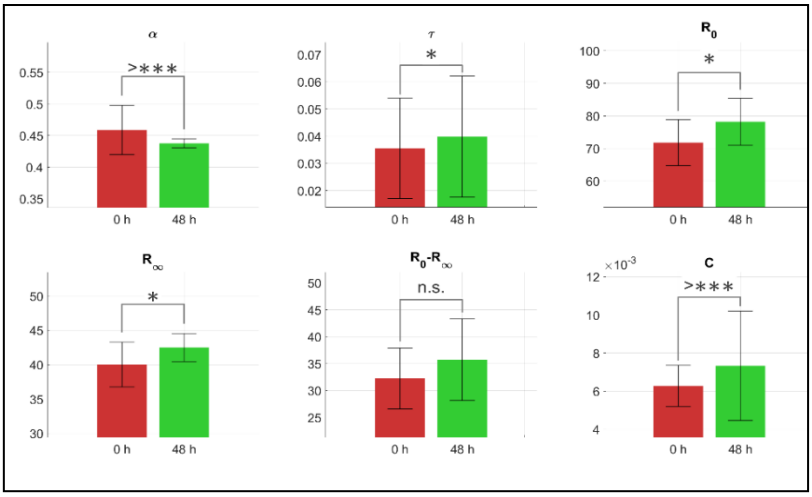
To study the effect of proliferation, a known number of cells were seeded onto graphene plates. After cell adhesion, a baseline BIS spectrum was recorded. Subsequently, the cells were incubated for an additional 48 hours to allow proliferation. After 48 hours, repeated BIS measurements were performed on the increased cell population.

To model changes in the extracellular space, the cell culture medium was replaced. Cells were seeded onto graphene plates, and BIS measurements were performed 24 hours after adhesion. Following the measurement, the exhausted cell culture medium was replaced with fresh medium, and the measurement was repeated.

The intracellular space was examined by inducing intracellular accumulation of polystyrene microspheres (PS-MS). To induce PS-MS accumulation, polystyrene microspheres (PS-MS) with a diameter of 0.8–1 µm were added to the cell culture medium at a concentration of 50 µg/ml after the cells had adhered, followed by an additional 24-hour incubation period. The PS-MS were taken up by the cells altering the electrical properties of the intracellular space. The intracellular uptake of the PS-MS was confirmed by flow cytometry.

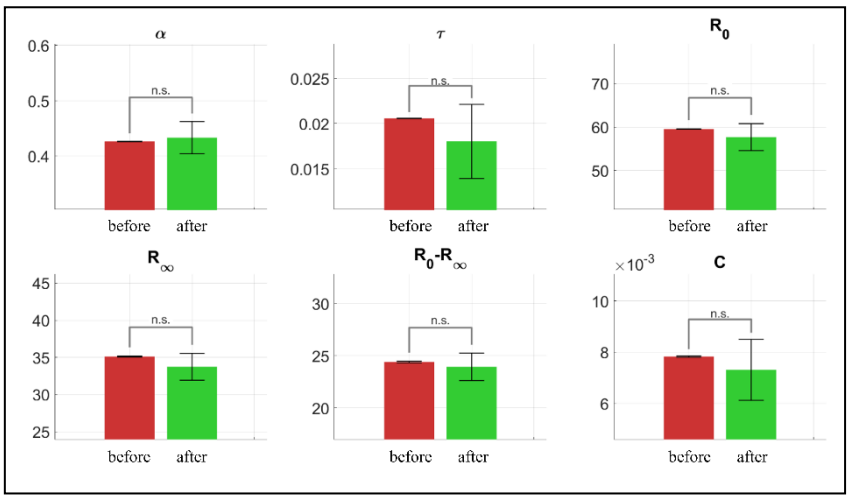
BIS results were evaluated using a single-dispersion Cole–Cole model. Comparing the changes in the R_0, $R\infty$, τ, α and C (capacitance) parameters calculated from the model made it possible to examine the correlation between the induced changes and cell biological characteristics.

The experiment examining the effect of cell division on Cole-Cole model parameters showed that changes in the α parameter and the capacitance (C) were the most significant in relation to the increase in cell number (Picture 3). The R_0, R_∞ and τ parameters showed s maller but still statistically significant changes.



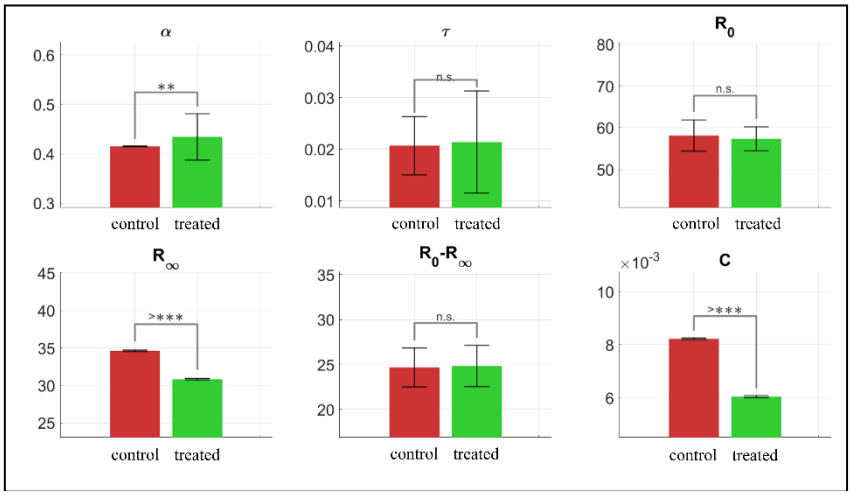
Picture 3. *Comparison of BIS results before (0 h) and after 48 h proliferation (48 h) based on Cole-Cole model parameters.*

Statistical analysis of the data collected for studying the extracellular space changes showed no significant differences in the model parameters before and after the cell culture medium change (Picture 4).



Picture 4. *Comparison of BIS results before and after the cell culture medium change based on Cole-Cole model parameters.*

Results from flow cytometry analysis showed that, on average, 40% of the cells uptake the PS-MS. Due to the intracellular accumulation of the PS-MS, significant differences were observed between the untreated (control) and treated groups for several model parameters (Picture 5).



Picture 5. *Comparison of the control and PS-MS-treated groups based on Cole-Cole model parameters.*

The most significant differences occurred in the $R\infty$ and the C (capacitance) parameters. A further, weaker significant difference found in the α parameter. There was no significant difference in the τ parameter between the untreated (control) group and the PS-MS-treated group.

3. Conclusions

In a series of in vitro experiments, Cole–Cole model parameters derived from BIS measurements were correlated with various physiological processes and cell biological characteristics. Experiments were designed to change only one factor at a time, thereby ensuring that the effects of individual factors could be examined separately and interpretable.

Analysis of the effect of cell division revealed a significant correlation between the α parameter and capacitance (C) with changes in cell number. However, the comparison between before and after the cell culture medium change did not result in a significant difference in any of the Cole-Cole model parameters, suggesting the self-developed BIS measuring method is not sensitive to changes in the extracellular space

under the presented conditions. With regard to changes induced in the intracellular space, the most significant differences between the untreater (control) and the PS-MS treated groups were observed in the R_∞ and capacitance (C) parameters, along with a slightly less pronounced difference in the α parameter. At low frequencies (R_0), the current flows through the intercellular space, whereas at high frequencies (R_∞) the current is able to penetrate the cell membrane; thus, the R_∞ parameter provides information about both the intracellular and extracellular spaces. Since the extracellular space (R_0) does not differ between the two groups (same cell culture medium), the strong, significant change observed in the R_∞ parameter likely reflects the change in the of the intracellular composition. Based on previous published data [10], accumulation of PS-MS within cells reduces cell proliferation but does not completely inhibit cell division; thus, the α parameter can be interpreted as a cell-number-sensitive parameter in the context of intracellular space study results.

In summary, it can be concluded that the self-developed BIS method is not sensitive to alterations in the extracellular space, indicating that the changes observed in the Cole–Cole model parameters in response to intracellular modifications can be attributed exclusively to changes in the internal environment of the cells. These results suggest that the self-developed BIS method is capable of detecting the effect of proliferation and changes in the intracellular space. With further research, the presented BIS measurement method could be suitable for the label-free, non-invasive monitoring of cell status and offers significant potential applications in medical diagnostics.

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