English
Research Express@NCKU Volume 23 Issue 1 - December 28, 2012
[http://research.ncku.edu.tw/re/articles/e/20121228/4.html]

A systematic investigation into the electrical properties of single HeLa cells via impedance measurements and COMSOL simulations

Min-Haw Wang and Ling-Sheng Jang*
Department of Electrical Engineering, College of Electrical Engineering and Computer Science, National Cheng Kung University

lsjang@ee.ncku.edu.tw

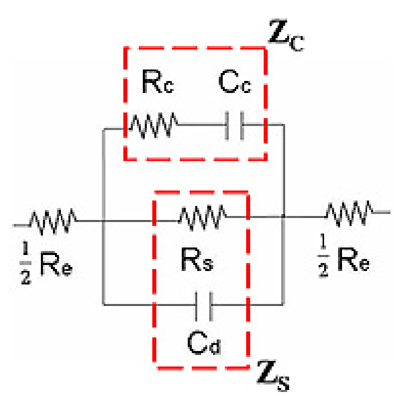
Biosensors and Bioelectronics, 24: 2830–2835, 2009
SCI Category: ELECTROCHEMISTRY, Ranking 1 /22 = 4.5 %

Introduction

In recent years, many researches have attempted to eradicate diseases in earliest stages. However, in the case of diseases such as cancer, conventional cell inspection techniques do not generally provide sufficient information to provide a reliable diagnosis since only a very small percentage of the cells exhibit symptoms of malfunction during the early stages of the disease's development. Moreover, in traditional inspection methods, the cellular parameters are average values and cannot exactly represent the characteristics of the individual cells. In addition, multiple parameters must be measured in real time in single living cells to correlate cellular events in order to develop a complete understanding of the complex cellular processes. Hence, single cell analysis is an emerging trend in the biological and medical research fields.

The present study performs a detailed experimental and numerical investigation into the impedance, permittivity and conductivity of single HeLa (human cervical epithelioid carcinoma) cells under a wide range of voltage and frequency conditions. In the experimental stage of the study, the electrical properties of the HeLa cells are investigated at voltages ranging from 0.1~1.0 V and frequency ranging from 1~100 kHz using a commercial impedance spectroscopy system integrated with the single-cell-capturing microfluidic device. The experimental results are then compared with the numerical solutions obtained from a model of the single-cell analysis system constructed using the commercial COMSOL software package. Finally, the simulation and experimental results are used to develop empirical expressions with which to predict the conductivity and permittivity of single HeLa cells at specified values of the operational frequency and voltage.

Theory and simulation



This paper develops a comprehensive electrical-biological-circuit-system comprising the cell impedance Z_c, the PBS solution impedance Z_s and the resistance of the electrode pair Re as presented in Figure 1. Here, Z_c comprises the capacitance of the cell membrane, C_{cell}, placed in series with the resistance of the cell cytoplasm, R_{cell}. Furthermore, Z_s represents the combined impedance of all the materials between the electrode pair, i.e. the resistance of the PBS buffer solution, Rs, arranged in parallel with the capacitance of the double layer, C .

Figure 1 The circuit diagram of the impedance model.

The COMSOL simulations consider a structure with overall dimensions of 100 μm x 100 μm supporting a gold (Au) electrode pair comprising two electrodes measuring 46 μm x 8 μm separated by a gap of 8 μm. In addition, the morphology of the cell is assumed to be spherical. Figures 2(a) illustrate the distributions of the electric field induced in the vicinity of the electrode pair for operational voltages of 0.1 V and 1.0 V. Note that the operational frequency is 1 kHz in both cases. Note also that the black circle in the figures represents a single HeLa cell with a radius of 10 μm located at the center of the electrode pair. It is observed that for both values of the operational voltage, the maximum electric field intensity is located toward the boundary region between the cell and the electrodes. Figure 2(b) plots the variation of the electric field strength along the X- axis direction for y=0 and at an operational voltage of 1.0 V. The greater rate of increase of the intracellular electric field relative to the extracellular electric field reflects the fact that the permittivity and conductivity properties of the HeLa cell are greater than those of the PBS solution.

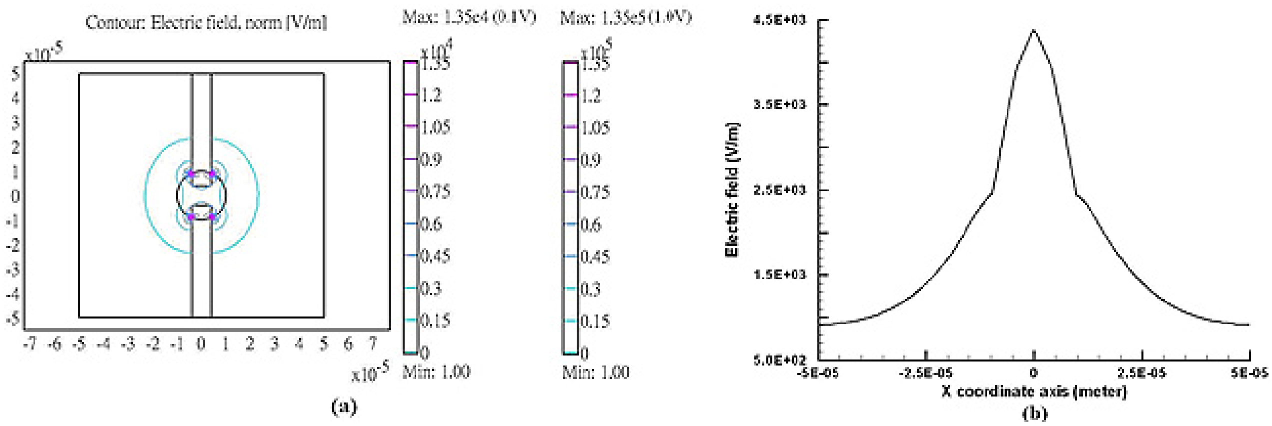


Figure 2 (a) Distribution of electric field intensity between electrodes for operational voltages of 0.1 V and 1 V. (b) Variation of electric field intensity in X-axis direction for operational frequency of 1 kHz.

Results and discussion

The solution of HeLa cells with a concentration of 2.25×10^5 cells/c.c was injected into the microfluidic channel using an infusion pump (KD Scientific Inc., KDS100) at a flow rate of 5 ml/h. The chips were visually inspected using an optical microscope (Nikon 50i) during the experimental process. Figure 3 shows the cell-trapped structures with and without the single HeLa cell. At the moment a single HeLa cell was trapped by the three micro-pillars, the infusion pump was turned off and the cell impedance was measured using a Precision Impedance Analyzer (Kayne Kerr Inc., 6440B).

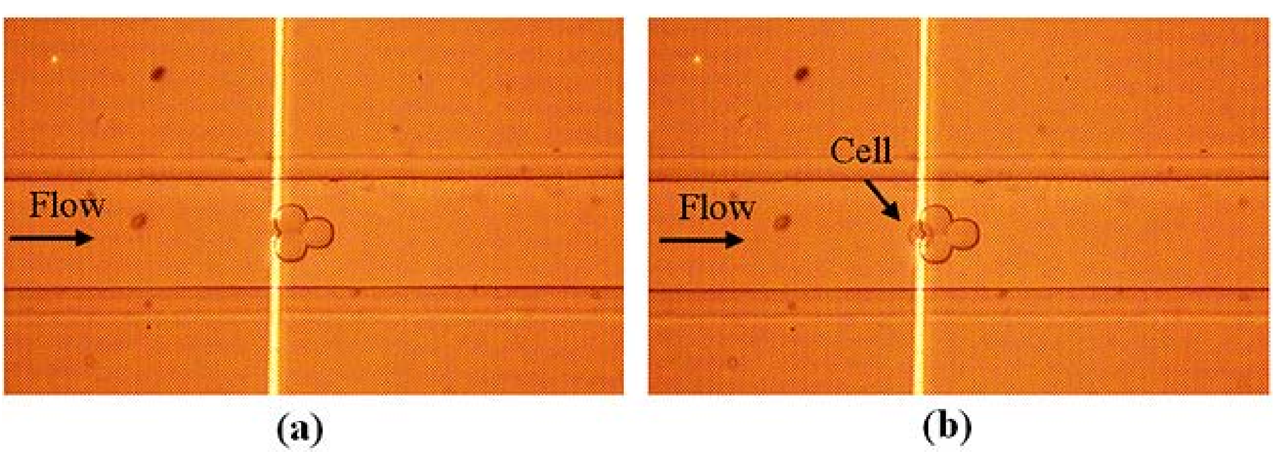


Figure 3 (a) The cell-trapped structure before injection of cell solution (b) the cell-trapped structure with a single HeLa cell after injection

Figures 4(a) and 3(b) illustrate the variation of the simulated values of the conductivity and permittivity of the single HeLa cell at operational frequencies ranging from 1.0 to 100 kHz and voltages in the range 0.1 to 1.0 V. The two figures show that both the conductivity and the permittivity of the HeLa cell increase with an increasing operational voltage at lower values of the operational frequency. This result suggests that the presence of a strong electric field opens the ionic channels of the cell membrane, thereby allowing a greater amount of current to pass through the cell. The higher permittivity of the HeLa cell under a stronger electric field is again thought to be the result of an opening of the ionic channels, which enhances the charge exchange between the cytoplasm within the cell and the isotonic solution surrounding the cell. In frequency domain, increasing frequency increases the conductivity. This result is to be expected since the current passes between the electrodes along the path of minimum distance at higher values of the operational frequency. Additionally, the relationship between the HeLa cell permittivity and the operational frequency is dependent on the value of the operational voltage. Specifically, at high operational voltages at 0.9 and 1.0 V, the permittivity of the cell reduces rapidly with an increasing frequency since the capacitance of the cell membrane cannot be fully charged within one cycle. At intermediate operational voltages in the range 0.6~0.8 V, the permittivity of the cell reduces slightly with an increasing frequency at operational frequencies greater than 30 kHz, but remains approximately constant at frequencies lower than 30 kHz. At low operational voltages of 0.1~0.5 V, the permittivity of the single HeLa cell remains constant at all values of the operational frequency since the capacitance of the cell membrane can be fully charged within one cycle.

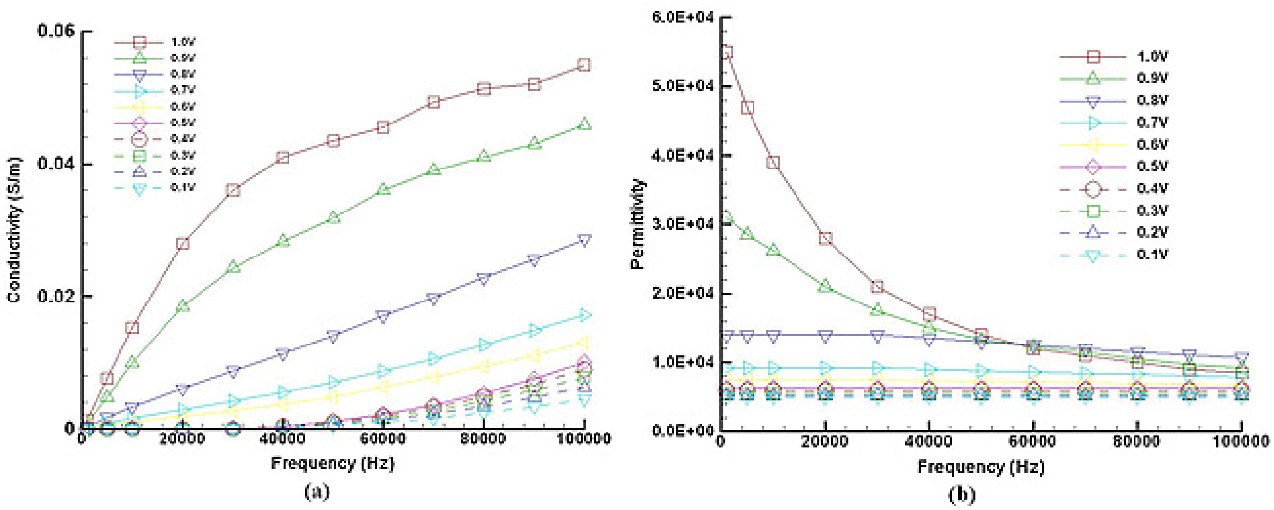


Figure 4 Variation of (a) conductivity and (b) permittivity of single HeLa cell with operational frequency as function of operational voltages in the range 0.1~1.0 V.

From the experimental results, we can see that the cell impedance varies with the operational voltages and frequencies. The simulation results show that the cell impedance is dependent on the cell conductivity and permittivity. According to the electroporation of the cell membrane, the parameters such as excitation voltage, frequency and ionic channel influence conductivity and permittivity of the cel. Cell electroporation is affected by some parameters including the interaction of the electric field with the lipid domains of the cell membrane, current, membrane conductance and transmembrane voltage. Therefore, we conclude that the cell conductivity and permittivity are function of the operational voltages and frequencies. Based upon the simulation results obtained for the conductivity and permittivity of the single HeLa cell, this study developed six empirical expressions to predict the conductivity and permittivity of single HeLa cells over the operational range of

0.2~1.0 V and 1~100 kHz. Figures 5(a) and 5(b) plot the experimental, simulation and prediction results for the variation of the magnitude and phase of the single HeLa cell with the operational frequency in the range 5~100 kHz at operational voltages of 0.2 V, 0.6 V and 1.0 V. It can be seen that the simulated and predicted results for the magnitude and phase are in good agreement with the experimental observations at voltages of 0.2 V and 0.6 V. However, at a higher voltage of 1.0 V, the predicted values deviate noticeably from the measurement results. The discrepancy between the two sets of results is most likely the consequence of a breakdown of the single HeLa cell under the effects of a high operational voltage.

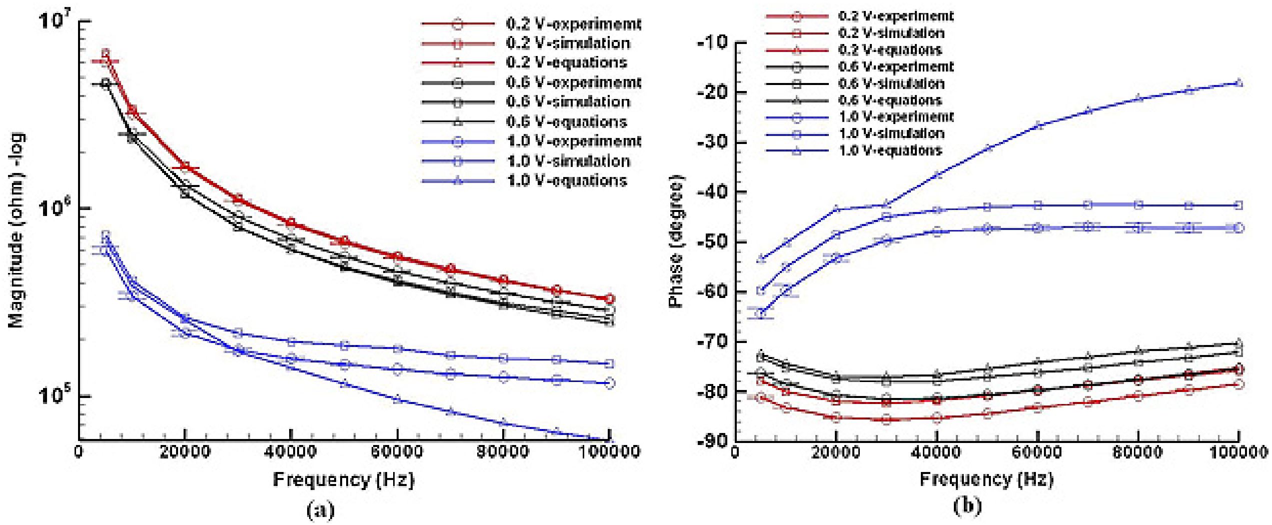


Figure 5 Variation of (a) magnitude and (b) phase of HeLa impedance signals as obtained experimentally, numerically and analytically for operational voltages of 0.2 V, 0.6 V and 1 V and operational frequencies in the range 5~100 kHz.

Conclusion

In summary, this study has performed an experimental, numerical and empirical investigation into the impedance properties of single HeLa cells. In the experimental stage, single HeLa cells were trapped in the microfluidic device and impedance properties were then measured at voltages ranging from 0.1~1.0 V and frequencies in the range 1~100 kHz using a commercial precision impedance analyzer. In the numerical stage of the study, an equivalent circuit model of the single-cell analysis system was constructed comprising a parallel arrangement of the cell impedance and the PBS solution impedance, respectively, configured in series with the impedance of a gold electrode pair. Utilizing the equivalent circuit model, COMSOL simulations were performed to investigate the magnitude and phase of the cell impedance under various values of the operational voltage and frequency, and to determine the corresponding values of the cell conductivity and cell permittivity, respectively. Based upon the simulation and experimental results, empirical expressions have been developed to predict the conductivity and permittivity of single HeLa cells for operational voltages in the range 0.2~1.0 V and frequencies in the range 5~100 kHz.