CONDUCTIVITY IN JURKAT CELL SUSPENSIONS AFTER ULTRASHORT ELECTRIC PULSING

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Abstract

Ultrashort electric pulses applied to similar cell lines such as Jurkat and HL-60 cells can produce markedly different results , which have been documented extensively over the last few years. We now report changes in electrical conductivity of Jurkat cells subjected to traditional electroporation pulses (50 μs pulse length) and ultrashort pulses (10 ns pulse length) using time domain dielectric spectroscopy (TDS). A single 10 ns, 150 kV/cm pulse did not noticeably alter suspension conductivity while a 50 μs, 2.12 kV/cm pulse with the same energy caused an appreciable conductivity rise. These results support the hypothesis that electroporation pulses primarily interact with the cell membrane and cause conductivity rises due to ion transport from the cell to the external media, while pulses with nanosecond duration primarily interact with the membranes of intracellular organelles. However, multiple ultrashort pulses have a cumulative effect on the plasma membrane, with five pulses causing a gradual rise in conductivity up to ten minutes post-pulsing.

Introduction

The plasma membrane of a cell separates the molecular contents of the cytoplasm from the external environment while the nuclear envelope performs an analogous role for the nucleoplasm in eukaryotic cells. Both the plasma membrane and the nuclear envelope can be considered as dielectrics that present a large energy barrier to transmembrane ionic transport, as well as gene and protein insertion into the cell. The outer barrier can be bypassed by applying short pulsed electric fields (PEFs), which permeabilize the membrane in a phenomenon called electroporation. Pulses of this type disturb the phospholipid bilayer of the plasma membrane, causing temporary aqueous pores to form. Generally, electroporation occurs for pulse widths on the order of 0.1-10 ms and electric fields on the order of a few kV/cm. Electroporation has a variety of applications, ranging from bacterial decontamination to medical treatments for cancer [1-2]. In recent years, research has been concentrated on using ultrashort PEFs with nanosecond pulse duration, opening a new frontier in cell research [3-5]. Decreasing the pulse width decreases the interaction of the pulse with the plasma membrane while increasing the interaction with intracellular structures, such as mitochondria and nuclei [6], with modeling studies carried out to further elucidate the mechanisms involved [7-8]. These intracellular effects raise the possibility that ultrashort electric pulses could be used to induce apoptosis in mammalian cells [9].

One way to explain PEF interactions with cells is through an electric circuit model [8]. This simplified circuit model considers those cells lacking a nucleus as a single-shell model while cells with a nucleus would be represented by a double-shell model [10]. Fig.1 shows a typical two-shell model for a eukaryotic cell, where ε and σ represent the permittivity and conductivity, respectively, of each structure within the cell. For maximum model accuracy, the electrical parameters must be known to determine the electrical potential distribution across the membranes. The lipid-containing cell and nuclear membranes are considered less conductive (dielectric) while the cytoplasm and nucleoplasm are more conductive due to abundant ions. However, measurements are difficult to conduct on a single cell, so the cells are often placed in a suspension. One technique used to provide ε and σ is TDS. Combined with sophisticated data processing and modeling [11-12], the parameters of the two-shell model shown in Fig. 1 can be obtained.

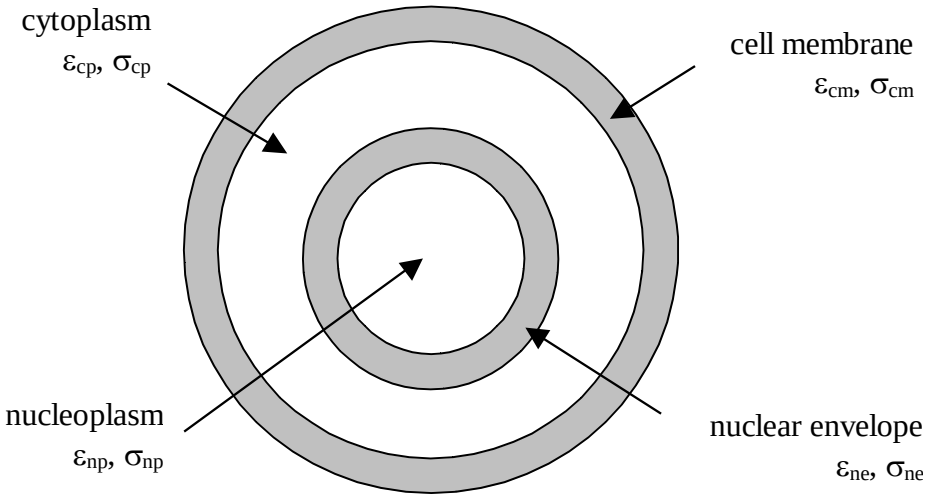


Fig. 1 Schematic picture of the two-shell model of a eukaryotic cell, where ε and σ represent the permittivity and conductivity, respecitvely.

TDS technique

The potential usefulness of TDS for broadband measurements at frequencies up to several gigahertz was recognized soon after significant development of fast pulse generators and sampling methods. Continuous improvement in electronics and data processing makes the technique an attractive method to monitor the dielectric behavior of biological materials. Fig. 2 illustrates the basic principle of TDS. Repetitive fast rising step voltage pulses generated by a tunnel diode are fed by coaxial lines through a sampling head to a sample (admittance termination).

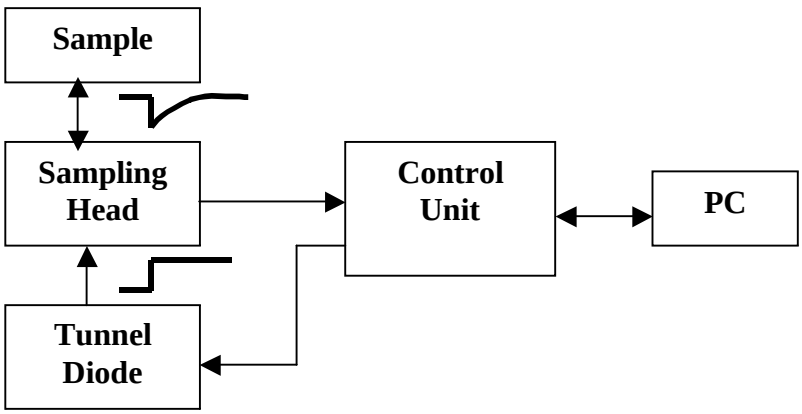


Fig. 2 Schematic diagram of TDS measurement.

The incident pulse and reflected pulses are then time stretched by the sampling circuitry. The output of the reflected signal contains the dielectric information of the sample. With calibration and data processing (Fourier transformation from the time domain to the frequency domain), one obtains the complex permittivity ($\varepsilon^*=\varepsilon'-j\varepsilon''$) of the sample. Details of the technique can be found elsewhere [13-14]. Fig. 3 shows a typical response from a biological system used in our experiment. In this case the reflected signal contains the information about the cell suspension.

By their nature, biological samples are conductive, which can mask the dielectric properties being sought due to the presence of a DC conductivity component and electrode polarization (the formation of a parasitic charge layer on the electrode surface). The TDS technique allows the measurement of the DC conductivity of the sample [14] while electrode polarization can be corrected analytically by considering the parasitic layer as an RC circuit. This report focuses on the changes in the conductivity of Jurkat cell suspensions subjected to microsecond and ultrashort PEFs.

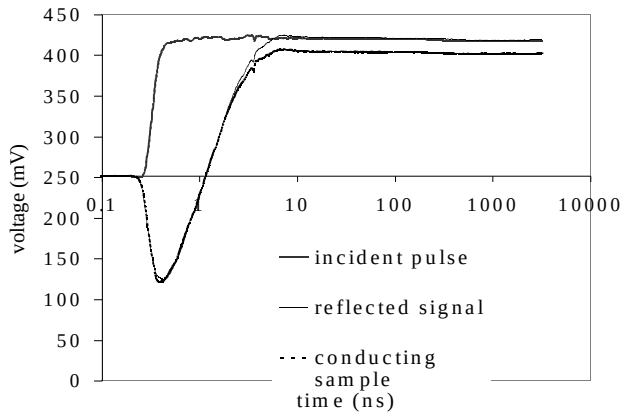


Fig. 3 Characteristics of a biological system.

Experimental

The cell suspensions to be pulsed were placed in gene pulser® cuvettes (BioRad) with an electrode distance of 1 or 2 mm. They were then exposed to either 2.12 kV/cm and 4.24 kV/cm pulses of 50 μs duration or 150 kV/cm pulses of 10 ns duration. The 50 μs was chosen to be roughly consistent with parameters used in typical electroporation experiments [15] while the 10 ns pulse was chosen to be consistent with previous intracellular pulsing research. The different field strengths permitted the short pulse to have approximately the same energy as the long pulse. A Blumlein pulse generator with an impedance of 10 Ω provided the 10 ns pulse with a rise and fall time of 1-2 ns. The 50 μs pulse was generated by the discharge of a capacitor. It was designed with a discharge time (RC time) much longer than the pulse length itself to provide a nearly constant voltage for 50 μs. The pulse length was regulated with a MOSFET, which allowed for a pulse rise time (and fall time) of 30 ns. The impedance of this system was also on the order of 10 Ω.

The Jurkat cell line used in our experiment is derived from human T-cell leukemia and this cell line is often used to determine the mechanism of differential susceptibility to anti-cancer drugs and radiation (America Type Culture Collection, Manassas, VA). The cells were cultured in RPMI-1640 Medium (ATCC), supplemented with 10% fetal bovine serum and 2% penicillin/streptomycin. Using 0.4% trypan blue (SIGMA), we measured cultured Jurkat cell viability to be greater than 80%. The cultured Jurkat cells were centrifuged three times for five minutes at 800 RPM at room temperature and then washed with sucrose/glucose buffer (229 mM sucrose, 16 mM glucose, 1 μM $CaCl_2$, and 5 mM Na_2HPO_4) in triple distilled water [12]. The cells were then adjusted to a 5% concentration.

The sucrose/glucose buffer was used to reduce the effect of electrode polarization, which could mask the true measurements of the cells and make it impossible to obtain reliable dielectric parameters. The buffer formulation allowed the gross morphology of the cells not to be altered for at least one and one half hours [12].

The cell concentration is also crucial to obtain accurate measurements. A low cell concentration makes the reflected signal too weak to provide adequate information about the cells [12]. As long as the cell concentration is kept below 20%, intracellular interaction is insignificant and the Maxwell mixture equation is valid [12]. From a practical standpoint, it is desirable to minimize the number of cells used because of the time required to grow them, so a cell concentration of 5% was arbitrarily selected for this study.

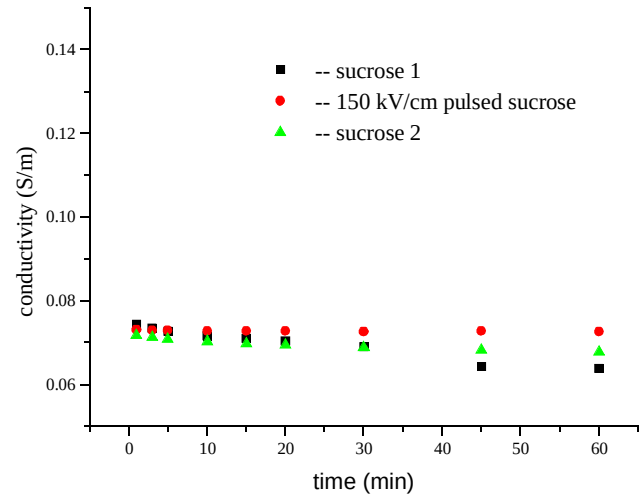
After cell preparation, we used the TDS system to measure dielectric properties of the suspension in a coaxial electrode system. To observe the effect of both microsecond and submicrosecond PEFs on the cells, we measured samples of buffer, pulsed buffer, cell suspension, and pulsed cell suspension. We conducted the measurements over a sixty minute period with typical measurements occurring at 1, 3, 5, 10, 15, 20, 30, 45 and 60 minutes. We conducted several measurements for the same sample type to verify repeatability.

Sufficient cell suspension was transferred to a gene pulser® cuvette (BioRad) for pulsing. After PEF application, we transferred a small amount to the electrode chamber for TDS measurement. The cell viability was checked occasionally to ensure that PEFs did not induce significant cell death. Because dielectric properties are sensitive to temperature, we maintained sample cell temperature at 25°C by using a thermostat (Julabo, US). In this report,

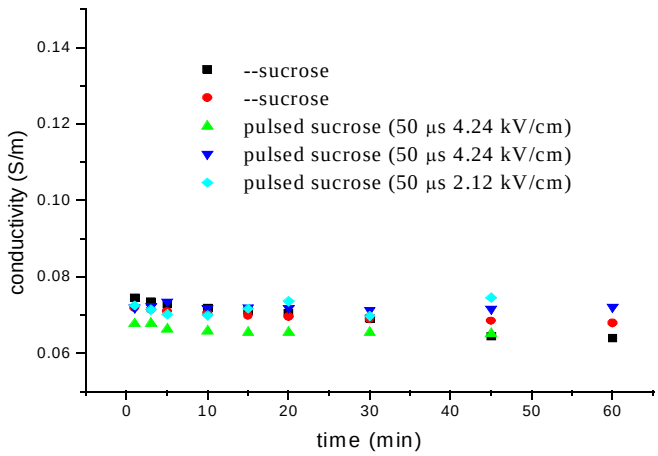
only the conductivity of the Jurkat cell suspension will be presented while the dielectric properties of the cells will be discussed in future publications.

Experimental Results

As a control, we monitored buffer conductivity prior to and after applying either a single 10 ns pulse or a single 50 μs pulse, with the results shown in Fig. 4. Fig. 4a shows a minor effect on buffer conductivity after applying a 10 ns, 150 kV/cm pulse. Fig. 4b shows that 50 μs pulses of either 2.12 kV/cm or 4.24 kV/cm have negligible effect on buffer conductivity. The triple distilled water used has a conductivity of ~10^{-6} S/m. Adding the sucrose/glucose buffer raises the conductivity to ~0.07 S/m. We observed slight changes in each set of conductivity measurements that are likely due to the calibration carried out each time and measurement errors. These results enable us to separate the effect of pulses on cells verses buffer in later experiments.



(a)

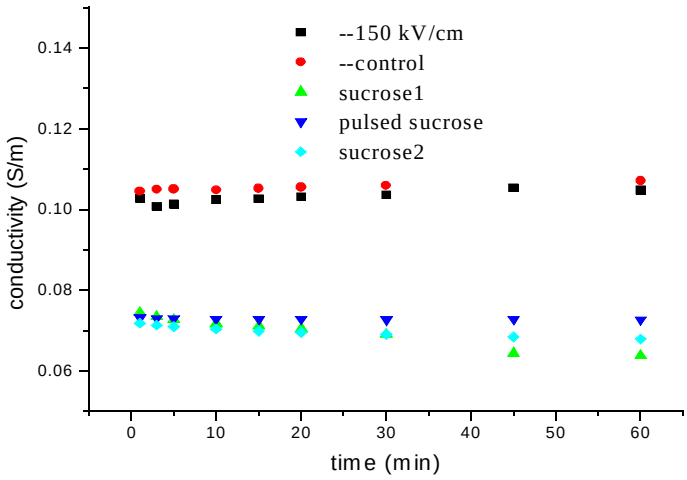


(b)

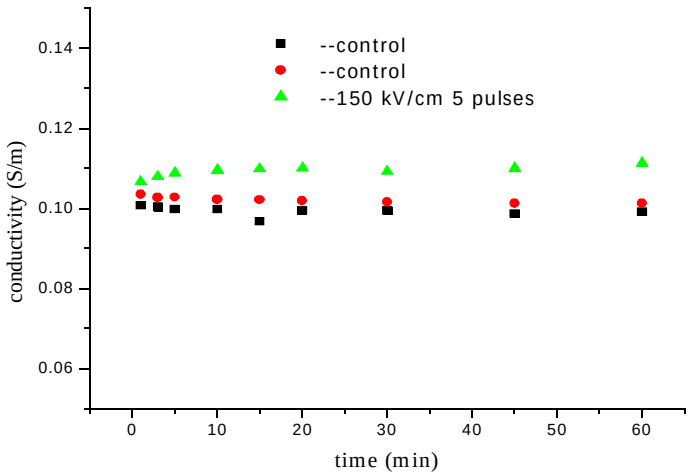
Fig. 4 Buffer conductivity after PEFs. (a) Applying a single 10 ns, 150 kV/cm PEF has little effect on buffer conductivity over a period of sixty minutes after pulse application. (b) Applying single 50 μs pulses of 2.12 kV/cm or 4.24 kV/cm have little effect on buffer conductivity.

Having established that buffer conductivity was not changed by either 50 μs or 10 ns pulses, we next measured cell suspension conductivity before and after applying either a single 50 μs pulse or a single 10 ns pulse. Fig. 5 compares pulsed and unpulsed cell suspensions with pulsed and unpulsed buffer. Adding Jurkat cells to the buffer significantly increased cell suspension conductivity even though the cells were washed three times by the buffer. One possible reason for the overall increase in suspension conductivity could be the Jurkat cell growth medium. This medium has a high conductivity due to ionic substances and washing three times may be

inadequate for completely removing the ions. An ion transfer process could also be taking place from cells to buffer due to an imbalance in the ion concentration across the membrane.



(a)



(b)

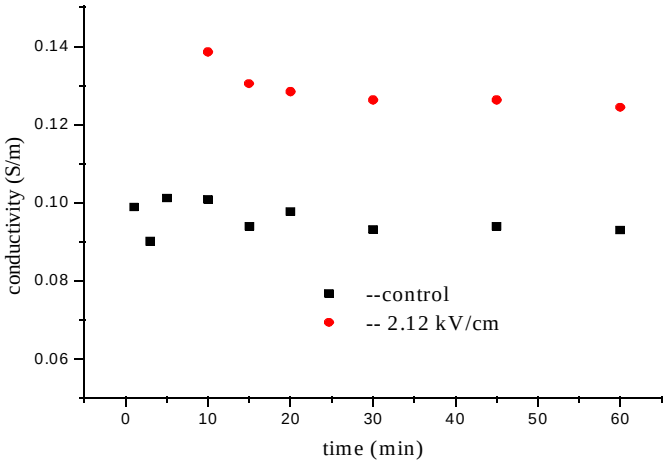
Fig. 5 Jurkat cell suspension conductivity following 10 ns, 150 kV/cm pulses. (a) A single pulse has a negligible effect on cell suspension conductivity. (b) Applying five pulses causes an initial rise in suspension conductivity followed by a plateau.

Fig. 5a shows a single 10 ns, 150 kV/cm pulse to have no noticeable effect on conductivity. Fig. 5b shows that applying 5 consecutive 10 ns, 150 kV/cm pulses to a cell suspension causes an initial increase in suspension conductivity before leveling off ten minutes after pulsing. The conductivity increase is not large, but distinguishable from the control samples.

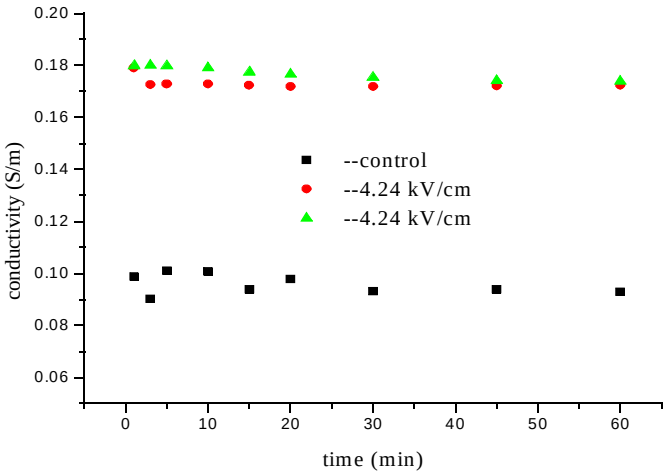
Fig. 6 shows cell suspension conductivity changes following a single 50 μs, 2.12 kV/cm pulse or a single 50 μs, 4.24 kV/cm pulse. Unlike the 10 ns pulses, the 50 μs pulses cause an obvious rise in suspension conductivity. The conductivity rise is more pronounced for the higher electric field (Fig. 6b) than for the lower one (Fig 6a), suggesting a stronger electric field induced larger change in the plasma membrane, possibly indicating cell lysis. Moreover, the conductivity increases immediately and has little variation over the measurement time for the higher electric field case.

Fig.7 shows conductivity measurements carried out on a cell suspension containing cells believed to be dead. The cell suspension was left in a tube at 37°C for 24 hours without CO_2 supplied. The viability examination

showed severe swelling and trypan blue uptake, indicating cell death. The conductivity of the suspension with dead cells was more than two times higher than the control sample.



(a)



(b)

Fig. 6 The conductivity of cell suspension after long pulses.

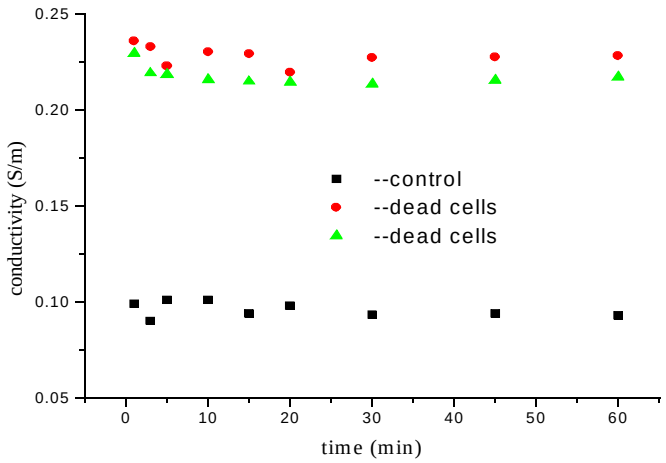


Fig. 7 Conductivity of cell suspensions containing dead cells.

One possible adverse influence on measurements is sedimentation of the Jurkat cells when the suspension is left in the sample for a relatively long period of time. However, our results indicate that the influence of sedimentation is unremarkable. Sedimentation of cells allows conductivity measurements to be performed on the overlying buffer that remains on top of the cell suspension after dead cells sediment to the bottom over a long period of time. The conductivity of the overlying buffer extracted from the suspension containing dead cells (without pulsing) was measured. Fig. 8 shows how the conductivity of the overlying buffer that was extracted from the cell suspensions used in Figs. 6 and 7 compares with the conductivity of dead cells and control buffer. Of particular note is that the conductivity of the extracted buffer is more than twice that of the dead cell suspension.

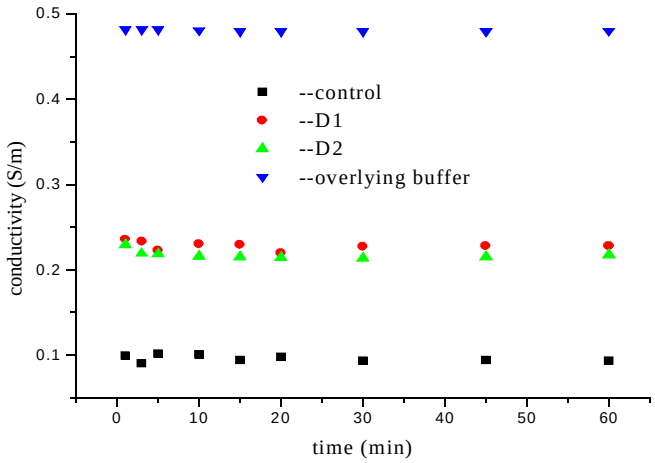


Fig. 8 Conductivity of the overlying buffer from the suspension used in Figs. 6 and 7 compared to that of dead cells (D1 and D2) and control buffer.

Discussion

From the measurements performed on the buffer, the conductivity of the buffer is evidently stable and does not show any effect of pulsing, regardless of pulse duration. This result was expected since any interaction between the PEF and the ions within the buffer will diminish rapidly. Over time, the buffer conductivity will only change if the ion concentration changes. While the PEF may stimulate ionization, the system should recover once the PEF is stopped given the short duration of the pulses and the relatively long timescale of measurements. As a result, the subsequent conductivity measurements should not reflect any consequence from the pulse field. This conclusion is important because any change in cell suspension conductivity after the PEF can then be attributed to the response of the cells to the pulses, although the initial conductivity of the suspension may affect the scale of the change [16].

Once Jurkat cells are added to the buffer, the conductivity increases by about 40% compared to the pure buffer with the exact amount depending on many factors, such as the cell preparation and cell concentration. Although we claim that the cell concentration is 5%, this is an estimated cell count. Typically, 10 μl of cell suspension was taken from the growth medium and stained for counting with trypan blue. The number of cells in the diluted solution were counted under the microscope and then multiplied by a scaling factor depending on the dilution of the suspension. Because the total number of cells is derived from a relatively small sample, a deviation from the claimed cell number may occur, leading to a slight variation in the conductivity from batch to batch. However, this variation is generally within 10%. The increase in conductivity after adding Jurkat cells has two likely reasons. First, the growth medium contains abundant ionic substances and washing three times with buffer may not be adequate for completely removing the ions, causing an increase in overall cell suspension conductivity. Second, ions may travel from the cytoplasm to the buffer due to the imbalance in ion concentration across the membrane, which causes ion concentration and conductivity in the buffer to rise. If the latter process is a dominant factor then the conductivity should show a time effect unless a balance is reached very quickly. Conducting measurements within fifteen minutes of cell suspension preparation and again an hour later showed that conductivity did not change dramatically, possibly indicating that the medium is the cause for the rise in suspension conductivity.

The effect of a single 10 ns pulse is not obvious, which is consistent with the current hypothesis that nanosecond pulses interact primarily with the membrane of inner organelles while leaving the cell membrane intact [17].

This means no significant ion exchange between cells and buffer occurred, causing no increase in cell suspension ion concentration or a concomitant rise in suspension conductivity.

On the other hand, applying five 10 ns pulses to a Jurkat cell suspension caused a rise in suspension conductivity, as shown in Fig. 5b. While the 10 ns pulse primarily interacts with the membranes of intracellular organelles, it is also likely that it interacts with the plasma membrane with less intensity. If enough 10 ns pulses are applied to the cell, the cumulative effect may be strong enough to electroporate the plasma membrane. Since the interaction with the plasma membrane is less effective than the interaction between the pulse and nuclear membrane, the pore opening is probably very small. Such an opening is thought to indicate a time delay [6]. The cells are able to recover and close the pores after a period of time. This may explain the observations in Fig. 5b. An initial rise in conductivity may indicate a delayed pore opening where the ions inside the cell leak out through the open pores. After ten minutes, the pores close upon full recovery of the plasma membrane. No more ions should traverse the membrane and enter the buffer as the cell would be trying to recover the lost ions. Consequently the conductivity stabilizes after 10 minutes. This accumulation effect has also been observed with 10 ns pulse above 100 kV/cm on sub-cellular functions [18].

Increasing the pulse duration causes the PEF-cell interaction to shift from the nuclear envelope to the plasma membrane. As discussed above, if PEF-plasma membrane interaction occurs, one would expect ions within the cell to leak out through the opened pores. We selected the 2.12 kV/cm and 50μs pulse because it has the same energy as the pulse of 150 kV/cm and 10ns and is likely to cause electroporation. The experimental results shown in Fig. 6a indicate that electroporation takes place fairly quickly without time delay. Increasing the pulse amplitude to 4.24 kV/cm causes the cell suspension conductivity to increase further, as illustrated in Fig. 6b. This higher conductivity likely indicates that the higher voltage causes stronger electroporation, thus permitting more ions to leave the cytoplasm and enter the buffer.

When the cell suspension is left for twenty-four hours without its required growing medium, the cells will gradually die. The integrity of the cells is destroyed and more ions will be released from the cells to the buffer. In this case, it is believed that the ions from both cytoplasm and nucleoplasm can leak from the cell into the buffer; therefore, the higher suspension conductivity observed in Fig. 7 is expected. The conductivity of the overlying extracted buffer (the top part of the cell suspension not containing the sedimented dead cells) is the highest. These results are reasonable since once the inside and outside of the cells achieve a balance, other substances within the cells are less conductive, and so the extracted buffer will be more conductive.

From these measurements, we can estimate the conductivity of the cytoplasm and nucleoplasm. Microscopic observation reveals the average diameter of Jurkat cells to be between 7 and 8 μm and the nucleus takes about 70 to 80 % of the cell volume. The high fraction of volume occupied by the nucleus is typical of lymphocytes and is retained in the cancerous cell line during their abnormal proliferation. We make the following assumptions to estimate the conductivity of the cytoplasm and nucleoplasm:

(i) the ratio of ion concentration within the nucleoplasm and cytoplasm is k,
(ii) the nucleus takes about 75% of the total cell volume,
(iii) all the ions have the same amount of charge q and
(iv) all the charges have the same mobility μ.

The last two assumptions are oversimplified, but necessary as an initial step to calculate the conductivities of the cytoplasm and nucleoplasm. In practice, it is more likely that different ions may possess different charge amounts. Because the conductivity is an average effect of the charge carriers and we are not interested in particular ions, this approach is acceptable. Based on these assumptions and two measurements made with the control sample and the extracted buffer, it is possible to calculate the conductivities of the cytoplasm and nucleoplasm. The conductivity, σ, of a material is generally given by

$$\sigma = qn\mu ,$$

where n is the concentration of charge carrier.

Assume that n_1 is the charge concentration in the control sample, n_2 the concentration in the extracted buffer, and n the concentration in the volume between plasma membrane and nuclear membrane of the cells. Furthermore, let V_1 represent the total volume occupied by the nuclei, V_2 the total volume of the cells, and V_3 the cell suspension. Thus, the relationships between the different volumes can be expressed as

$$\frac{V_2}{V_3} = 0.05 \quad \text{and} \quad \frac{V_1}{V_2} = 0.75$$

while the carrier concentrations are related to different volumes, i.e. n_1 is related to V_3-V_2, n_2 to V_3, n to V_2-V_1 and kn to V_1. Therefore, the relationship among the different concentrations can be expressed by

$$n_2 = 0.95n_1 + 0.05 \times 0.25n + 0.05 \times 0.75kn .$$

Based on the conductivity equation, the following relationship can be established:

$$\frac{\sigma_1}{0.95n_1} = \frac{\sigma_2}{n_2} = \frac{\sigma}{n} .$$

The cytoplasm conductivity σ can be expressed in terms of the measured σ_1 and σ_2 as

$$\sigma = \frac{\sigma_2 - \sigma_1}{0.05(0.25 + 0.75k)} .$$

The conductivity of the cytoplasm depends on the measured values for σ_1 and σ_2 as well as the ion concentration ratio *k*, which may be influenced by many factors, such as the specific type of cell being studied. K values from 0.6 to 0.83 have been obtained for lymphoblast [10]. If we take an average value of k = 0.71, then we obtain conductivities of 9.712 S/m and 6.896 S/m for the cytoplasm and nucleoplasm, respectively. However, recent work indicates that k = 2 [12]. If this value is used, the conductivities for cytoplasm and nucleoplasm are 4.343 S/m and 8.686 S/m, respectively, which are on the same order as those published for other cell types. The values estimated from our conductivity measurements are slightly higher. Apart from the assumptions made being a source of error, some of the ions may not participate in the conduction process when the integrity of the cells is intact. Thus, the current method overestimates the ion concentration, leading to an upper limit of the conductivities for cytoplasm and nucleoplasm.

Summary

The changes in electrical conductivity of Jurkat cell suspensions subjected to ultrashort and microsecond PEFs have been investigated. The conductivity was measured using the TDS system and the following conclusions may be drawn from the research.

The conductivity does not show noticeable change following a single short pulse with a magnitude of 150 kV/cm and width of 10 ns. We observed larger changes for a 50 μs pulse with a magnitude of 2.12 kV/cm that had the same energy density as the 10 ns pulse. The results support the hypothesis that the short electric pulse interacts primarily with the nuclear membrane while the longer pulse interacts with the plasma membrane, causing ion leakage and a concomitant rise in cell suspension conductivity. Applying multiple 10 ns pulses causes a cumulative effect on the plasma membrane, with five 10 ns pulses causing a gradual rise in suspension conductivity for up to ten minutes following PEF application. Increasing the field strength of 50 μs pulses further increases the suspension conductivity after PEF application because of increased poration of the cell membrane allowing more ions within the cell to leak across the plasma membrane into the buffer.

The conductivity of the suspension containing dead Jurkat cells supports the ion leakage explanation. More ions leak into the buffer when the cells are dead since the ions from both the cytoplasm and nucleoplasm are available. Compared to electroporation, where only ions from cytoplasm are available, the conductivity of the cell suspension containing dead cells is higher.

The conductivity of the extracted buffer from the suspension containing dead Jurkat cells is particularly interesting. As the plasma membrane and nuclear membrane are dielectric (having low conductivity values), the extract shows the highest conductivity as expected. More importantly, based on this value and the conductivity of the control cell suspension, it is possible to estimate the conductivities of the cytoplasm and nucleoplasm. The conductivity values obtained are slightly higher than those in the literature for other cell types, but on the same order. The current method overestimates the ion concentration participating in the conduction process, therefore, it is likely to give an upper limit.

Acknowledgments

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