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Analysis of the Electric Field-Dependent Current During Electroporation Pulses

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ABSTRACT When delivered across a cell, certain pulsed electric fields can cause an increase in the cell membrane permeability through a biophysical process known as electroporation. The current signals during the electric pulses could be used as a method for noninvasive electroporation measurement because of the sharp change in the conductivity of cells due to electroporation. To add to the existing knowledge on electroporation current signals under different pulse parameters, we undertook a study in which the electric current across the cells was recorded during electroporation pulses. The experimental current response to a pulsed electric field consisted of three stages: a) a rapid initial increase followed by b) an exponential decrease and then c) a monotonic increase. The rise time of the current signals was not affected by the intensity of the electric field or the number of pulses. However, the time at which the current increased again, deemed the electroporation onset time, shortened as the electric field became more intense and as the number of pulses increased. The transient conductivity change rate, defined to describe the electroporation degree during the pulse, increased under a higher electric field strength. However, the transient conductivity change rate first decreased and then gradually increased with additional pulses. This work may provide insight into the change in current during real-time electroporation detection.

INDEX TERMS Electroporation, current signal, pore initiation time, conductivity change.

I. INTRODUCTION

Electroporation is used widely in biological research and for medical applications [1]. In electroporation, cells are exposed to a pulsed electric field to change the structure of the cell membrane to markedly increase membrane permeability [2]. There is general agreement in the literature that the electric field creates pores in the cell membrane, which significantly increases the transport of ions and molecules across the cell membrane. This is why the process is termed electroporation [3]. The term “electropermeabilization” is also used to describe this process and emphasize the increased cell permeability. For certain electric fields, after the electric field has ceased, the pores (permeabilization) may persist for a few seconds to a few minutes, and then the cell returns to

its original state. This process is known as reversible electroporation [4] and is exploited for the uptake of drugs or genetic material into cells [5], the insertion of proteins into the cell membrane [6], and the fusion of individual cells with tissues [7]. However, cells may succumb to permanent permeabilization of the cell membrane when the electric field is sufficiently high or applied for a long duration. This phenomenon is called irreversible electroporation (IRE) [8] and is used for bacterial inactivation [9], tumor ablation [10], and food processing [11].

In general, the experimental research on electroporation includes investigations of single cells and cell suspensions as well as in vivo experiments [12], [13]. An important research topic is studying the pores (permeabilization) during electroporation. Lee *et al.* [14] showed numerous, well-circumscribed, round, and concave pore defects disturbing the hepatocyte plasma membranes after electric field

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application using scanning electron microscopy (SEM). These pores were not seen in normal liver cells. A study involving molecular dynamics simulations also showed evidence of pore formation in the lipid bilayer of the cell membrane [8]. However, the direct observation of poration may be difficult due to the time scale limitation of imaging measurements. Some researchers have used indirect measurements to detect the increased membrane permeability due to different electrical pulses. Measurements using optics and dyes support the claims of pore formation and development [15]–[17]. For instance, the uptake of dyes, such as propidium iodide (PI) and YO-PRO-1, has been used to evaluate poration and to study the effects of different pulse parameters during and after electroporation [16], [18], [19]. Another area of research on cell membrane permeabilization during electroporation employs measurements of the transmembrane voltage across the cell membrane [22]–[24].

Recently, sharp changes in the dielectric properties of cell membranes have been found to reflect alterations in membrane permeability and have been used for the real-time noninvasive detection of electroporation processes [25]–[29]. The cell membrane can be simplified as an equivalent circuit with parallel conductance and capacitance. Electroporation changes the cell membrane permeability and causes an immediate increase in cell membrane conductivity, thereby immediately affecting the measured impedance of the cell suspension during or after the application of a pulsed electric field [25]. Dunki-Jacobs *et al.* [27] found that the mean change in tumor tissue resistance and the slope of the resistance curve could be used intraoperatively to evaluate the success of tumor ablation during IRE treatment. The dielectric properties of a cell, such as conductivity, can also be evaluated from simple current-voltage recordings during the application of an electric pulse, and important research was published on the evolution of currents during electroporation investigated using various cell and tissue models. An increase in the current through a cell suspension during the application of an electroporation pulse has been measured and described [25]. Pavlin and Miklavcic [29] measured the voltage and current of electric pulses in high-density cell suspensions. These researchers found that the changes in current reflected the changes in dielectric properties, and they discussed the relationship between the short-lived transient pores and the long-lived pores based on the current-time signature during the pulse.

Electric current signals can be used to noninvasively probe changes in the dielectric properties of the cell membrane in real time and, thus, can be used to detect cell electroporation during a pulse. We believe that an understanding of the electroporation process can be gained from experiments that measure electric currents during electroporation and from further analysis of these experimental data. For this purpose, we performed a series of electroporation experiments with a dense suspension of mouse melanoma cells (B16F1). We measured the electric currents as a function of time under various electric field

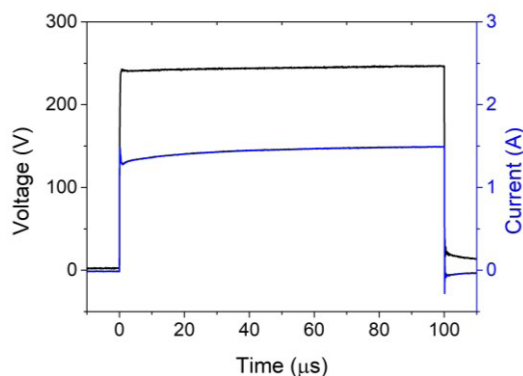


FIGURE 1. Voltage and current waveforms for an electric pulse of 240 V (1200 V/cm).

strengths and numbers of pulses to analyze the current signals.

II. METHOD

A. EXPERIMENTAL PROTOCOLS

An electroporation generator designed with 4 MOSFET switches in series to deliver square electrical pulses was developed in the laboratory. This generator can produce constitutive pulses with an amplitude from 0 to 3000 V. Eight 100- μ s pulses with an interval time of 1 s were used as a prototype, and an electric field between 600 V/cm and 1800 V/cm was applied to analyze the changes in the current waveform. During the pulse, the output electric voltage and current were measured and stored using a WavePro 760Zi-A oscilloscope (Teledyne LeCroy Inc., New York, USA) with a PPE-5 kV high-voltage probe and a Pearson current probe 6600 (Pearson Electronics Inc., Palo Alto, USA). The sampling frequency of the oscilloscope was set to 100 MHz. The temporal data were analyzed using MATLAB software. The voltage and current waveforms for a pulse of 240 V recorded during experimentation are shown in Figure 1 as an illustration.

Cell suspensions of mouse melanoma cells (B16F1), which were donated by the Southwest Hospital of the Third Military Medical University, China, were used in all of the experiments. The cells were propagated at 37 °C with 5% CO₂ in air in DMEM (Gibco) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Before the experiment, the cells were washed with 1–2 mL phosphate-buffered saline and digested with 0.5 mg/mL trypsin. After 2 min, the trypsin and growth medium were removed from the obtained cell suspension by centrifugation at 1000 rpm, and new DMEM was added. Note that the current increase due to electroporation is most readily observed in dense cell suspensions and tissues in which cells represent a large part of the sample volume [30]. Therefore, dense cell suspensions with a density of 1.0×10^8 cells/mL were prepared. A small aliquot (50 μ L) of the cell suspension was injected into a 2-mm gap cuvette (Harvard Apparatus, Holliston, MA) for electroporation. For every set of parameters, reference measurements on the DMEM without cells were also performed.

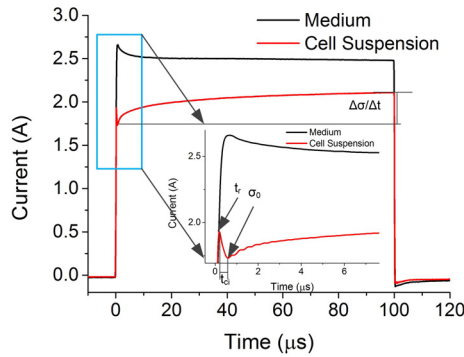


FIGURE 2. Current signals from DMEM alone and a cell suspension under a 1500 V/cm electric field.

The data are presented as the mean ± standard deviation for three independent experiments for each protocol.

III. RESULTS

Figure 2 shows the current signals from DMEM with and without cells during an electric pulse. Rems [31] noted that an electric field directly generates forces that tend to move charged ions and molecules and to orient the permanent and induced electric dipoles in the medium. Initially, the electric dipoles polarize rapidly due to the rapid rise in the applied electric field, which results in a dramatic initial current increase (Figure 1). However, when the applied electric field reaches a constant value, the polarization then gradually reaches equilibrium, slowing the change in the current [32]. Therefore, when an electric field is applied to the DMEM without cells, the current initially increases and then quickly decreases exponentially until it stabilizes (Figure 2). When a pulsed electric field is applied to the cell suspension, the current initially increases and then quickly decreases exponentially, similar to the pattern for DMEM alone; however, at this point, the current starts to increase again with the cell suspension before stabilizing, which is different from the pattern with DMEM alone. This feature is the key signature of cell membrane electroporation.

Figure 2 also illustrates the typical parameters that we use to analyze the current signals. The rise time t_r is the time between the onset of the pulse application and the time the peak current is reached. The time t_{ci} represents the time between when the peak current is reached and when the current begins to increase again. From the measured voltage and current waveforms, we obtained the conductivity of each cell suspension sample as $\sigma(t) = I(t)/U(t) \cdot d/S$, where $I(t)$ is the current, $U(t)$ is the voltage, d is the distance between the electrodes and S is the area of the contact surface between the sample and the electrodes. We define the transient conductivity change rate $\Delta\sigma^N/\Delta t^N$ during the n th pulse as the difference between the final and initial conductivity values, which is then normalized to the time between t_{ci} and the end of the pulse ($\Delta t = 100 \mu s - t_{ci} - t_r$). The initial conductivity is defined as the conductivity when the current starts to increase again during the pulse.

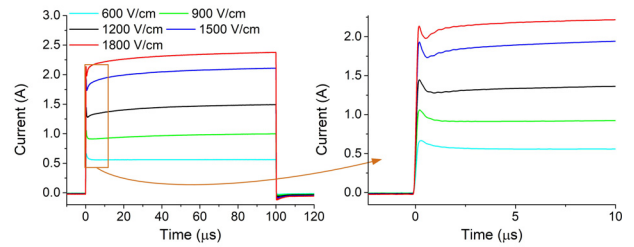


FIGURE 3. Measured current waveforms for cell suspensions under increasing electric field strength and an enlarged view of the 0-10 μs window.

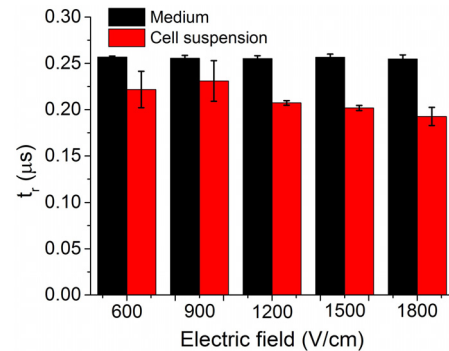


FIGURE 4. Rise times t_r of the currents for cell suspensions and medium under increasing electric field strength.

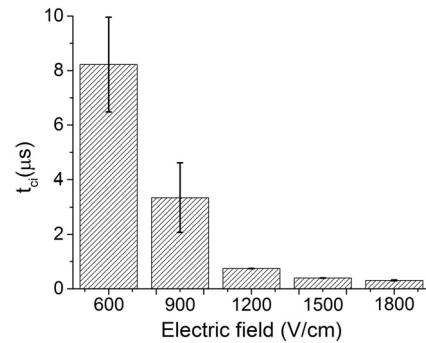


FIGURE 5. Time t_{ci} when the measured current starts to rise again under increasing electric field strength.

Figure 3 displays the measured current signals from a cell suspension under different applied electric fields. The measured current signals start to increase again during the pulse from 600 to 1800 V/cm. Figure 4 show the rise time in the current signal under fields of different strengths. The rise time of the current in the cell suspension and medium t_r is not significantly different under each of the applied electric fields. This suggests that this part of the current evolution curve is related to the intrinsic capacitance of the system rather than the electroporation process. Figure 5 shows that t_{ci} decreases as the electric field increases. For an electric field of 900 V/cm, the time at which the current starts to increase again t_{ci} is 3.34 μs. At 1200 V/cm, t_{ci} decreases to 0.74 μs, and at 1800 V/cm, t_{ci} decreases to 0.31 μs. A higher-strength electric field could make the time at which the current increase again shorter, which indicates that electroporation in the cell membrane may occur earlier at a higher

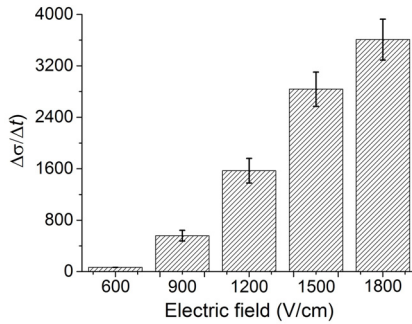


FIGURE 6. Measured transient conductivity change rate $\Delta\sigma/\Delta t$ under increasing electric field strength.

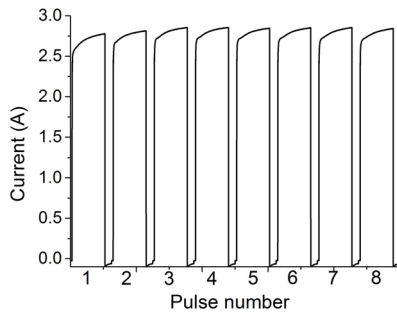


FIGURE 7. Recorded waveforms of the measured current during a train of eight 100- μ s pulses with an interval of 1 s for an applied electric field of 1800 V/cm.

electric field strength. Figure 6 shows the transient conductivity change rate $\Delta\sigma/\Delta t$ under different electric fields. The transient conductivity change rate increases as the electric field strength increases. However, for electric field of 600 V/cm, the transient conductivity change rate does not increase obviously, which is also shown in Figure 3.

Figure 7 demonstrates the current signals after different numbers of pulses. The current continues to undergo an increase as the pulse number increases, which indicates that the electroporation degree may continuously increase as the number of pulses increases. However, Figure 8 shows that the rise time t_r remains constant as the pulse number increases, which further indicates that this part of the current evolution curve is related to the capacitance of the cell suspension. The time at which the current starts to increase again t_{ci} gradually decreases with increasing pulse number (Figure 9). For the electric field of 600 V/cm, the mean value of t_{ci} for the first pulse is longer than that for the second pulse, and t_{ci} then increases slightly with additional pulses. For an electric field above 900 V/cm in intensity, the t_{ci} for the second pulse is shorter than that for the first pulse, but the t_{ci} tends to plateau with additional pulses. For instance, the t_{ci} is 0.40 μ s for the first pulse and 0.34 μ s for the second pulse under an electric field of 1500 V/cm. The t_{ci} for the second pulse is significantly shorter than that for the first pulse ($p = 0.0011$). However, the t_{ci} is 0.33 μ s, 0.33 μ s, and 0.31 μ s for the third, fifth, and eighth pulses, respectively. After the second pulse, the t_{ci} slightly decreases with additional pulses.

Figure 10 presents the transient conductivity change rate $\Delta\sigma^N/\Delta t^N$ during a pulse as a function of the pulse number.

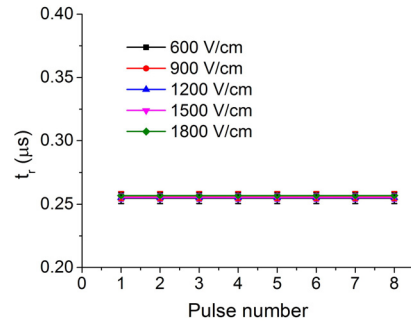


FIGURE 8. Rise times t_r of the currents for cell suspensions with an increasing number of pulses under electric fields of different strengths.

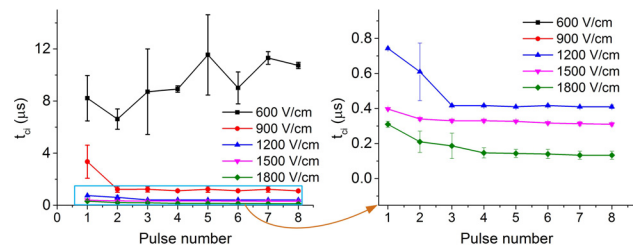


FIGURE 9. Time t_{ci} at which the measured current starts to rise again for an increasing number of pulses under electric fields of different strengths.

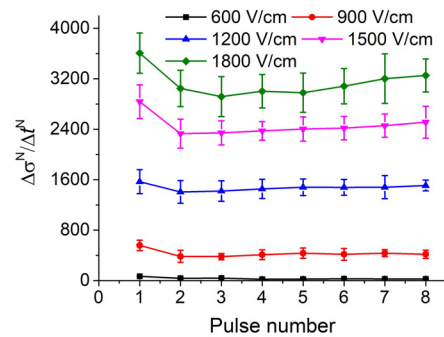


FIGURE 10. Measured transient conductivity changes $\Delta\sigma^N/\Delta t^N$ with an increasing number of pulses under electric fields of different strengths.

The transient conductivity change rate for the first pulse is larger than that for the second pulse and then slightly increases with an increasing number of pulses when the electric field strength exceeds 900 V/cm. For example, under an electric field of 1500 V/cm, $\Delta\sigma^N/\Delta t^N$ is 1405.854 S/μ s for the second pulse, which is smaller than that for the first pulse (1569.390 S/μ s), and $\Delta\sigma^N/\Delta t^N$ is 1419.876 S/μ s, 1481.254 S/μ s, and 1508.554 S/μ s for the third, fifth, and eighth pulses, respectively. After the second pulse, $\Delta\sigma^N/\Delta t^N$ slightly increased with additional pulses. Figure 11 shows the change in conductivity between the pulses $(\sigma^N - \sigma_0)/\sigma_0$ as a function of pulse number. Clearly, the relative change in conductivity between the pulses is larger at higher pulse numbers and eventually plateaus with increasing pulse number.

IV. DISCUSSION

Controlling and analyzing the current waveform during cell suspension pulses could be considered a noninvasive method for the real-time detection of electroporation processes.

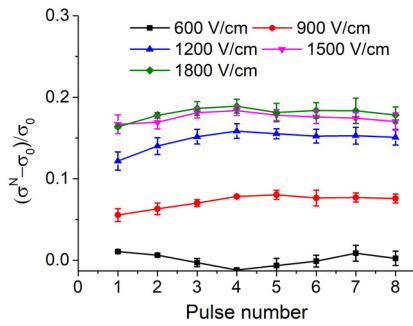


FIGURE 11. Measured conductivity changes $(\sigma^N - \sigma_0)/\sigma_0$ with an increasing number of pulses under electric fields of different strengths.

The current across the cells contains information on the electroporation process. The current could experience a second increase during the pulse due to the electroporation (Figure 2). Since an electroporation model containing an asymptotic Smoluchowski equation (ASE) is commonly used to simulate cell electroporation progress [21], [33]–[35], we preliminarily attempted to model the electric current across an electroporated cell (details are provided in the supplementary materials).

The above model is used to simulate the electric current when the electroporation is allowed or when it is not allowed (Supplemental Figure S2). When electroporation is not allowed to occur, the current initially increases rapidly and then decreases before leveling off during the pulse. However, when electroporation is allowed to occur, the cell membrane undergoes electroporation under a pulsed electric field before the current has reached a steady state, causing a significant increase in cell electroporation. This simulated current trend is similar to the experimental current (Figure 2). Moreover, the simulated currents also experience a second increase during the pulse with the electric field between 600 V/cm and 1800 V/cm (Supplemental Figure S3). Therefore, the time at which the current starts to increase again during the pulse may be considered the beginning of electroporation.

Figure 9 shows that the time at which the current starts to increase, t_{ci} , is earlier under electric fields of higher strength at any pulse number. In particular, the t_{ci} for the second pulse is smaller than that for the first pulse under an electric field above 900 V/cm. After the second pulse, the t_{ci} shows a slight decrease with an increasing pulse number. The electric pulse could create pores in the cell membrane. However, some pores may reseal and return to their normal status after the electric pulse has ceased. When subsequent pulses act on the cells, these resealed pores in the cell membrane reopen. Then, t_{ci} is determined mainly by the onset time of electroporation. Given that t_{ci} can be considered the onset of pore opening, it shortens after more pulses have been applied.

Electroporation, which causes an immediate increase in cell membrane conductivity, could provide more pathways for electric current across the cell. Therefore, the transient conductivity change rate $\Delta\sigma/\Delta t$ used in this study is defined to describe the electroporation degree after the onset of

electroporation. The transient conductivity change rate during the pulse is larger under electric fields of higher strength. Interestingly, the transient conductivity change rate $\Delta\sigma^N/\Delta t$ first decreases and then rises slightly with additional pulses (Figure 10). Since the cell membrane can be deemed an equivalent circuit with parallel resistance and capacitance, its conductivity is relatively low (5×10^{-7} S/m). When electroporation in the cell membrane occurs, the conductivity of the cell membrane sharply increases during the first pulse. After the first pulse, some pores may reseal. However, some pores may still exist and the cell membrane cannot return to its original status before subsequent pulse is applied [31], which may be the reason that the transient conductivity change rate $\Delta\sigma^N/\Delta t$, defined to describe the electroporation degree change rate during the n th pulse, increases but not as sharply as it did during the first pulse. Successively, the transient conductivity change rate $\Delta\sigma^N/\Delta t$ gradually increases with additional pulses. However, the number of pulses does not significantly influence the transient conductivity change rate.

Figure 11 shows that the conductivity change $(\sigma^N - \sigma_0)/\sigma_0$ between pulses increases as the number of pulses increases, as additional pulses could have an accumulative effect that increases the electroporation degree. However, saturation is reached above a certain number of pulses. Pavlin and Miklavcic [29] used voltage and current signals to discuss the relation between short-lived and long-lived pores. They believed that short-lived pores could be formed rapidly during the pulses and then disappear quickly when the pulse ceased. Therefore, the application of new pulses is necessary to reopen the pores in the cell membrane. Furthermore, we infer that a certain number of short-lived pores are transformed to long-lived pores, indicating that the conductivity increases with additional pulses. Therefore, the conductivity change between pulses $(\sigma^N - \sigma_0)/\sigma_0$ increases with additional pulses.

This study mainly investigated the current waveform of a cell suspension based on the electroporation process. Electroporation is considered the main cause of the second current increase during a pulse. The experimental analysis in this study provides valuable insight into the real-time detection of electroporation. First, the experiments and analysis revealed that the initial increase and subsequent decay of the current during electroporation are not entirely related to the electroporation process itself but are rather systemic behaviors of a system that is a composite of capacitance and resistance elements. Second, the effects of electroporation dominated the last stage of the current signature, namely, the sequence when the current increases again during the application of the electroporation electric field. The time at which the current starts to increase again during a pulse can be considered the time of initial pore opening. Moreover, the transient conductivity change rate can be used to analyze the electroporation degree under different pulse parameters after the onset of electroporation.

Furthermore, this study surprisingly found that the currents simulated using a common electroporation model also experience a second increase during the pulse. Therefore,

the electroporation model containing an ASE could be used in further research on the current waveform for real-time electroporation detection. However, the simulated currents do not match the experimental currents quantitatively, and there may be several reasons for this. First, a multicellular environment with arbitrary shapes, arrangements and orientations may have a considerable effect on the electroporation process and thus affect the current signal [35]–[38]. Second, not all of the parameters required by the model for a single cell type were available in the literature. Finally, Pavlin *et al.* [39] and Susil *et al.* [40] have found that the actual local field is smaller than the applied field due to the interaction between cells in suspension.

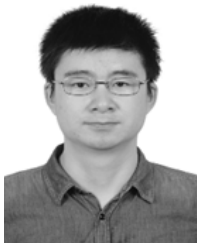
V. CONCLUSION

In this study, currents were measured experimentally across a dense cell suspension during the application of various electric pulses. The time at which the currents increased for the second time, considered the time of initial pore opening, occurred earlier at a higher electric field strength and after a high number of pulses. The transient conductivity change rate increased under higher electric field strength. However, the transient conductivity change rate first decreased and then gradually increased with additional pulses. This study may provide valuable insights into the use of current signals for real-time electroporation detection.

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