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14. ABSTRACT

Experimental and modeling studies have shown that nanosecond electric fields allow us to affect internal structures of biological cells. Electrical diagnostics work with temporal resolution in the sub-nanosecond range is critical for evaluation of such ultrafast pulses for the extension of this new bioelectric technique from presently used electrode application in cell suspensions and tissues, to electromagnetic field interactions using antennas. Experiments are ongoing using the 4 GHz bandwidth oscilloscope purchased by this grant for studies on pulsed electron heating in plasmas and electrical breakdown of liquids. Another purchase in support of bioelectrics research is a Time Domain Dielectric Spectroscopy (TDDS) system. It allows us to measure the complex dielectric constant of biological suspensions, and via modeling, get information on cell membrane resistivities and capacitances. A TDDS-system, developed specifically for biological studies, has been purchased from IDC Expertise LDC, and has recently generated the first results on the temporal development of the electrical parameters of HL60 cells in pulsed electric fields.

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ABSTRACT

Results of experimental and modeling studies have shown that nanosecond electric fields allow us to reach internal structures of biological cells. The research effort at the Center for Bioelectrics at Old Dominion University has focused on exploring these bioelectric effects for pulses in the temporal range of one nanosecond. A 4 GHz bandwidth oscilloscope, purchased with funding from this grant, has allowed us to evaluate the electrical parameters of ultrashort pulses with risetimes in the 100 ps range. Measurements in this temporal range are critical for extending this new bioelectric technique from presently used electrode application to cell suspensions and tissues to electromagnetic field interactions using antennas. Besides for biological studies, experiments are underway to use the 4 GHz bandwidth oscilloscope for studies on pulsed electron heating in plasmas and electrical breakdown of liquids. A second piece of equipment that was purchased in support of bioelectrics research is a Time Domain Dielectric Spectroscopy (TDDS) system. It allows us to measure the complex dielectric constant of biological suspensions, and through modeling, get information on cell membrane resistivities and capacitances. A TDDS-system, which has been developed specifically for biological studies has been purchased from IDC Expertise LDC, and has just recently generated the first results the temporal development of the electrical parameters of HL60 cells in pulsed electric fields. The system is expected to become a standard tool for bioelectric studies on cells and tissues.

SUMMARY

Experimental studies on the effect of ultrawideband electrical pulses on eukaryotic cells have been performed in an Air Force Office of Scientific Research supported project *(AF F49620-99-1-0069)* by research teams at Old Dominion University and Eastern Virginia Medical School, and is now the topic of an AFOSR directed MURI on "Subcellular Response to Narrowband and Wideband Radio Frequency Radiation *(F49620-01-1-0506)*" and an AFOSR project on Bio-Inspired Concepts *(F49620-01-1-0506)*. The results of these studies have confirmed the hypothesis that short electrical pulses (short compared to the charging time constant for the outer cell membrane) allow us to affect intracellular structures [1], and that they induce apoptosis in cells [2]. This is quite different from the effect of longer, multimicrosecond pulses, which only cause electroporation. The newly discovered electric field – cell interactions, which we have termed "Intracellular Electromanipulation" promise to have therapeutic applications, such as tumor treatments.

Intracellular electromanipulation is the prime research area where the two systems, which have been purchased through this DURIP program (4 GHz Oscilloscope and a Time Domain Dielectric Spectroscopy system), are being applied. However, the use of the 4 GHz oscilloscope is not restricted to bioelectrics research. High speed diagnostics is also required in two other AFOSR funded projects where plasma generation in gases (*AFOSR F49620-00-1-0079*) or liquids (*F49620-01-1-0354: prime agreement, administered through the University of New Mexico, E. Schamiloglu, P.I.*) are studied. The electron kinetics in these plasmas change on the time scale of nanoseconds and less, making extreme high-speed diagnostics a necessity.

4 GHz Oscilloscope (TDS7404)

Application in Bioelectrics

Modeling results have shown that reducing the pulse duration, or risetime, respectively, allows us to affect increasingly smaller substructures. The extension of our research, which has initially focused on studies in the 10 ns range, to the 1 ns range and even shorter, allows us to explore the electrical effects on such structures as mitochondria The mitochondrion seems to play a major role in the observed apoptosis of human cells, a programmed cell death [2]. Besides the pulse duration, the risetime of the electrical pulse is possibly of importance for the stimulation of apoptosis.

In order to explore the effects in the temporal range of 1 ns, we had earlier purchased a 1 ns, 350 kV, 50 Ω pulse generator from Applied Physical Electronics, L.C. with funding provided by Old Dominion University under a special program in bioelectrics. The risetime of the generated pulse into a biological load is an important

parameter, since it defines the high-frequency component of the pulse. With the new oscilloscope we were for the first time able to measure the temporal development of the pulse with a resolution in the 100 ps range. A voltage trace measured at the output of the pulse generator [Fig. 1] shows that the output pulse of the generator with a risetime of 600 ps. The ability to measure with such high temporal resolution has opened the field of bioelectrics to the subnanosecond range, an important range for exploring wideband electromagnetic effects on cells.

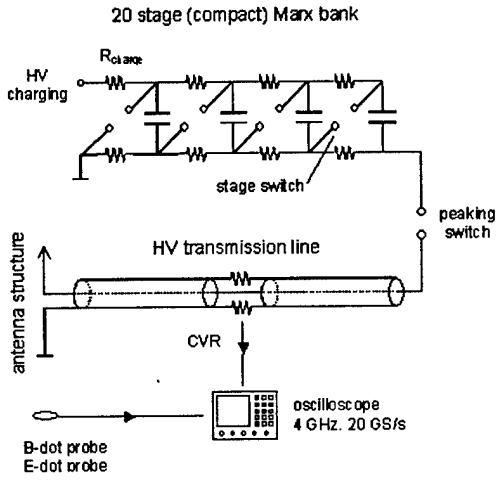

Figure 1a. Circuit diagram of the 350KV, 50 Ω, 1 ns pulse generator

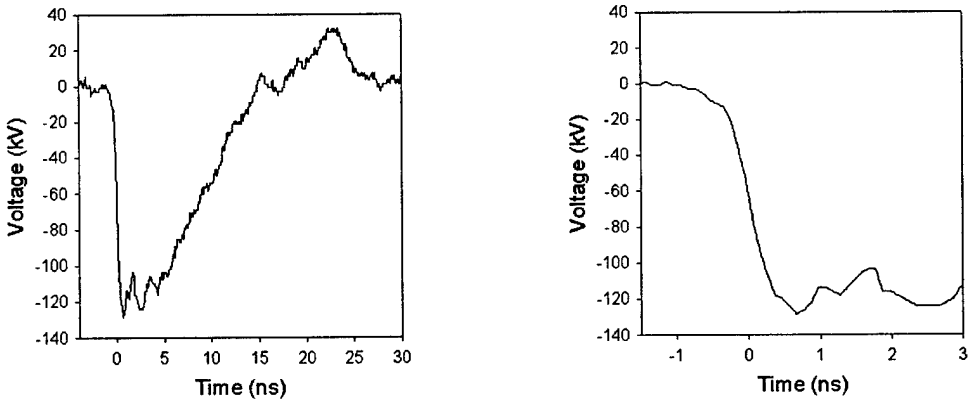

Figure 1b. Voltage traces observed with the 4 GHz oscilloscope showing pulse rise times of 600 ps (right figure detail).

Applications in Plasma and Electrical Discharge Science

Other applications of the newly acquired 4 GHz oscilloscope include measurements of the electrical parameters of plasmas which undergo pulsed electron heating (*AFOSR F49620-00-1-0079*), and studies on the electrical breakdown in liquids (*F49620-01-1-0354*). The first topics deals with nanosecond pulsed electric fields applied to plasmas, a technique which has been shown to heat the electron temporally without

heating the plasma [3]. This strongly nonlinear increase in electron energy, is being used to reduce the power consumption for electromagnetic plasma mirrors or ramparts, and for chemical and bacterial decontamination. The electron kinetics changes on the time scale of nanoseconds and less, making extreme high-speed diagnostics a necessity. Electrical breakdown of liquids, also requires diagnostics with high temporal resolution. Electrical diagnostics is an important tool for the measurements of nonequilibrium processes in plasmas, independent of the medium used to generate them.

A. Electron Heating by Means of Pulsed Electric Fields

Weakly ionized plasmas, generated in high pressure glow discharges, are able to reflect or absorb electromagnetic radiation in the microwave range and consequently act as temporally controllable barriers for this radiation: as plasma ramparts. At equilibrium conditions, where the electron energy distribution is determined only by the value of the reduced electric field, the power density required to sustain an atmospheric pressure air plasma of 10^{13} cm^{-3} electron density is approximately 5 kW/cm^3, a value which makes these equilibrium plasmas impractical for applications as plasma ramparts. Shifting the electron energy distribution to higher values than determined by the static electric field, promises to increase the electron gain processes and reduce the electron loss processes in the plasma, and consequently to reduce the power consumption. Such conditions can be achieved by heating the electrons with wideband electrical pulses.

The application of nanosecond voltage pulses to weakly ionized atmospheric pressure plasmas allows heating the electrons without considerably increasing the gas temperature [1]. Measurements of the temporal development of the voltage across an atmospheric pressure glow discharge in air and the optical emission in the visible after applying a 10 ns high voltage pulse showed an increase in plasma decay time from tens of nanoseconds to microseconds, and consequently a reduction in power consumption by more than two orders of magnitude. Even stronger reduction can be achieved if the electrical pulse is shortened to approximately 1 ns. We have a 1 ns high voltage pulse generator, obtained from university funds, which provides a 350 kV pulse into a 50 Ω load. But we have not yet used it for this project, because of the lack of adequate electrical diagnostics. The acquisition of the 4 GHz bandwidth oscilloscope from Tektronix (TDS7404) will allow us to extend our research by one order of magnitude with respect to temporal resolution.

B. Ultrafast Electrical Breakdown in Liquids

Research on liquids as switching and energy storage media at Old Dominion University is funded through a MURI on Portable Pulsed Power, administered by the U. New Mexico. The potential dielectric strength of liquids allows us, when used in switches, to reduce the gap even for voltages of 100 kV, to values on the order of 1 mm. Small size gaps favor high speed operation, since the inductance of the switch can be

minimized. In addition, the liquid can be flown through the switch gap, removing the ions and debris produced during the discharge, and making high repetition operation possible.

Experiments have shown that pulse risetimes of nanoseconds and possibly less can be achieved [4]. Measurements of the changes in electrical parameters on this time scale require electrical diagnostic systems with temporal resolution of less than one nanosecond. Again, as for electron heating of weakly ionized gases, the TDS7404 allows us to explore energy transfer (breakdown) processes in liquids.

Time Domain Dielectric Spectroscopy System

Specificity in the response of cells to ultrashort pulses is important for applications of intracellular electromanipulation [1], as responses vary, depending on cell type. One way to predict differences is based on the different electrical parameters such as cut-off frequencies for transmission of electromagnetic radiation through membranes and membrane charging time constants, respectively, for cells. The membrane charging time constants are determined by the capacitance and resistance of the membrane(s) and the resistivity of the cell plasma.

A method to obtain information on electrical parameters of cells is Time Domain Dielectric Spectroscopy (TDDS), based on the pioneering work of Fricke [5]. The Time Domain Dielectric Spectrometer (TDDS) is based on transmission line theory in the time domain. It utilizes the reflected and transmitted signals from and through the load, respectively. It allows us to determine the dielectric properties of cells in a suspension by measuring the response of a sample to a rapidly increasing electrical pulse.

A research team at the Hebrew University in Jerusalem, Israel, under the guidance of Prof. Yuri Feldman, has developed a TDDS system for biological applications. It allows for accurate measurements of the complex dielectric constant of cells in suspensions [6]. This system provides cell data, which allow us to tailor pulse shapes towards controlled electric field-cell interaction. It also provides information on temporal changes in cells affected by pulsed electric fields. This information is not only of scientific value, but might also lead to therapeutic applications. Recent measurements with Time Domain Dielectric Spectroscopy have e.g. shown that differences between healthy and malignant cells exist [7]. Based on these differences selective absorption, and consequently selective cell lysing might be possible.

In order to operate the system which was purchased from an UK company which builds such systems (based on Feldman's research) we have requested guidance by the inventor by Prof. Feldman. He has provided this help generously, by a) visiting the Center for Bioelectrics in Fall of 2002, and instructing the student working on this topic in the use of the TDDS. B) In addition, he has allowed the student to work for one week with his team at the Hebrew University, in Fall of 2002. The team of Prof. Feldman continuoes to provide help in evaluating the data obtained with the system.

Experimental studies on HL60 cells using the TDDS system have already been presented at the 2003ElectroMed in San Antonio [8], and have resulted in a conference paper [9], which has been accepted for presentation at the 3003 IEEE Conference on Dielectric and Electrical Insulation in Albuquerque, NM, in October of 2003. Both the abstract of the ElectroMed presentation and the conference paper is enclosed.

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[9] Allen L. Garner, Jing Yang, Nianyong Chen, Juergen Kolb, Karin C. Loftin, R. James Swanson, Stephen Beebe, Ravindra P. Joshi, and Karl H. Schoenbach' "Effects of Electrical Pulses on the Dielectric Properties of Biological Cells," to appear in the Proc. of CEIDP, Albuquerque, NM, October 2003.

Symposium Record, Abstracts, ElectroMed2003, San Antonio, TX, June 11-13, 2003

Effects of Electrical Pulses on the Dielectric Properties of Cancerous Cells

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Abstract

A fundamental part of biological cells, the cell membrane (biomembrane) serves a variety of functions, including separating cells, transporting water-soluble molecules by embedded protein transporters, and housing receptor proteins that bind circulating signaling molecules as they travel from cell to cell. Despite the basic knowledge of biochemical processes, the electrical properties, particularly the change in membrane properties upon the application of pulsed electric fields (PEF's), have not yet been fully characterized. PEF's above a certain threshold result in the phenomenon of electroporation, or the significant increase in the cell's permeability due to the formation of pores within the cell membrane. Electroporation has many practical applications, including gene therapy, decontamination, electrochemotherapy, food decontamination, and transdermal drug delivery. Another potential application is to use PEF's as a direct means to selectively destroy cancer cells rather than just facilitating drug introduction as in electrochemotherapy. As a first step to assessing the feasibility of this application, we use the technique of time domain dielectric spectroscopy (TDDS), a non-intrusive technique that measures the reflection of a small electrical pulse to determine the conductance and permittivity of a cell suspension. In this experiment, we use TDDS to measure the electrical properties of the HL-60 and healthy cells prior to PEF application and then compare these results to those obtained at various times after PEF application. The measurements elucidate the mechanisms which PEF's cause in the cells and provide a means for determining the efficiency of using PEF's to selectively destroy cancerous cells.

To appear in the Proc. of CEIDP, Albuquerque, NM, October 2003.

Effects of Electrical Pulses on the Dielectric Properties of Biological Cells

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Abstract: Despite a basic knowledge of cells' biochemical processes, their electrical properties, particularly the changes in membrane properties upon the application of pulsed electric fields (PEF's), have not yet been fully characterized. Microsecond pulses above a certain threshold cause electroporation of the cell membrane while nanosecond pulses of higher voltage additionally porate the inner organelles. We used Time Domain Dielectric Spectroscopy to measure the conductivity of HL-60 (human leukemia) cell suspensions as a function of time after 10 ns, 78.5 kV/cm pulses and 50 μs, 1.1 kV/cm pulses, which have the same energy. The conductivity increased immediately after the 50 μs pulse, indicating that ion channels in the HL-60 membranes initially opened. However, the conductivity decreased immediately after the ultrashort pulse, indicating that ion channels initially closed. The conductivity decreases significantly approximately 40 minutes after both pulses. This suggests that not only do the pores or channels opened close, but pores or channels open in the membrane prior to the pulse may close as well. These measurements were an intermediate step in determining the electrical properties of HL-60 cells using a two-shell model. Once determined, these electrical parameters will be used in electroporation models developed at Old Dominion University.

Introduction

A fundamental part of biological cells, the cell membrane (plasma membrane) serves a variety of functions, including separating cells, transporting water-soluble molecules by embedded protein transporters, and housing receptor proteins that bind circulating signaling molecules as they travel from cell to cell [1]. PEF's above a certain threshold cause electroporation, or the significant increase in the cell's permeability due to the formation of pores within the cell membrane [2]. Electroporation has many practical applications, including gene therapy, decontamination, and transdermal drug delivery [3], [4]. Moreover, shorter pulses of higher voltages affect the membranes of the inner organelles and can cause apoptosis, with potential application in cancer treatment [5]. Despite these numerous applications, the basic mechanisms are not well understood and modeling of intracellular processes is still in an infant stage [2].

Figure 1 shows the two-shell model of a typical, eukaryotic cell with the cell membrane as the outer surrounding shell and the nucleus as the inner shell. This model is used in many electroporation models [6] and in Time Domain Dielectric Spectroscopy (TDDS) to obtain the electrical parameters of the different parts of the cell [7], [8]. For electroporation models using the two-shell model, one must know the electrical properties of the cell membrane, cytoplasm, nuclear envelope, and nucleoplasm [1], [6].

Using TDDS allows the measurement of these parameters for various cell types under different conditions. Feldman's group has observed that the dielectric permittivity, capacitance, and conductivity of the cell membranes and nuclear envelopes of healthy lymphocytes were significantly higher than for malignant ones [8]. They have also shown that external factors can impact the dielectric properties of cells, such as their study of the effects of glucose on the dielectric properties of erythrocytes [9].

In addition to providing the necessary electrical data for electroporation models, TDDS is a non-intrusive technique that provides some insight into cellular mechanisms based on the changes in electrical parameters. In particular, understanding how the electrical properties of the cells change after pulse application could lead to insight into how electroporation pulses change the outer membrane [2], [3], [4] and ultrashort pulses alter cell functions [5].

Experimental Setup and Protocol

TDDS System

Figure 2 shows a block diagram of the Time Domain Spectrometer (IDC Expertise, United Kingdom) based on Feldman's design [7]. Because of the strong sensitivity of dielectric measurements to temperature, we used a thermostat (Julabo, US) to maintain the temperature of the sample cell at 25 C to be consistent with the TDDS literature [7]-[9].

The basic concept of the TDDS technique is to apply a small pulse to a cell suspension and use a sensitive diode to measure the reflection of this signal to

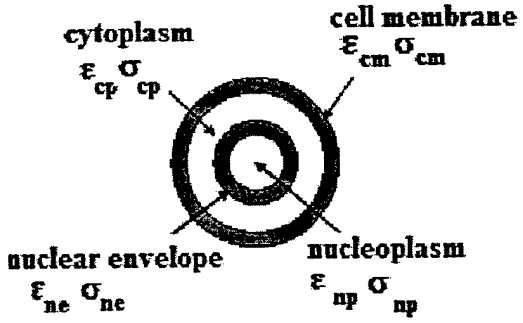

Figure 1: Schematic picture of the two-shell dielectric model of the cell from [8], where ε is permittivity and σ is conductivity. This model is used in the theoretical analysis of electroporation [6] and in determining the dielectric parameters of eukaryotic cells in a cell suspension [8].

obtain the electrical properties of the cell suspension, as shown in Figure 3. An external pulse generator provided a 200 mV, 5 μs pulse with a risetime of 40-50 ps. A key advantage of using TDDS is that one can obtain the dielectric spectrum of the sample over a wide frequency range by using only a *single* pulse [7].

To convert the reflected signal of the suspension into a dielectric measurement, we measure the reflected signals of open and short circuit terminations as a calibration. Data analysis software (IDC Expertise) can then be used to determine the permittivity and conductivity of the sample by comparing the reflection of the baselines to the reflection of the sample [7]. Once obtained, the permittivity and conductivity of the cell membrane, cytoplasm, nuclear envelope, and nucleoplasm can be obtained using the two-shell model in Figure 1 [8].

Pulser Design

The cell suspensions to be pulsed were placed in gene pulser® cuvettes (BioRad) with an electrode distance of 2 mm. They were then exposed to either 1.1 kV/cm pulses of 50 μs duration or 78.5 kV/cm pulses of 10 ns duration. The 50 μs was chosen to be roughly consistent with parameters used in typical electroporation experiments [2]-[4] while the 10 ns pulse was chosen to be consistent with the intracellular pulses and have approximately the same energy as the longer pulse [5]. The 10 ns pulse was provided by a Blumlein pulse generator with an impedance of 10 Ω. The applied pulse had a rise- and fall time of 1-2 ns, limited by the employed spark gap. 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.

Cell Suspension

We used HL-60 cells, a human promyelocytic leukemic cell line (America Type Culture Collection, Manassas,VA), in our experiments. 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 the viability of the cultured HL-60 cells to be greater than 80%. The cultured HL-60 cells were centrifuged twice for five minutes at 700 RPM at room temperature. We washed them once with phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+} and once with a buffer consisting of 229 mM sucrose, 16 mM glucose, 1 μM $CaCl_2$, and 5 mM Na_2HPO_4 in double distilled water [8]. The cells were then adjusted to a 5% concentration.

In our initial experiments, we used Hank's Balanced Salt Solution (HBSS) for the buffer; however, the conductivity of this buffer was significantly higher than the sucrose buffer because salts are the primary constituents vice sucrose and glucose. This caused electrode polarization, which masked the true measurements of the cells and made it impossible to obtain accurate dielectric parameters [8]. Using this glucose buffer did not alter the gross morphology of the cells for at least one hour [8]. We noted that the viability of the cells in sucrose/glucose buffer was comparable to those in HBSS over the hour and a half of measurements.

The cell concentration is also crucial to obtaining accurate measurements. A low cell concentration makes the reflected signal too weak to provide adequate information about the cells [8]. As long as the cell concentration is kept below 20%, intracellular interaction is insignificant and the Maxwell mixture equation used in the TDDS software (IDC Expertise, United Kingdom) is valid [8]. From a practical standpoint, it is desirable to minimize the number of cells used because of the time required to grow them, so we use a cell concentration of 5%.

Protocol

Once the cells were prepared, we measured the TDDS spectrum of a control (unpulsed) sample. We then transferred sufficient cell suspension to a gene pulser® cuvette (BioRad) for pulsing. We applied either a single 1.1 kV/cm, 50 μs pulse or a 78.5 kV/cm, 10 ns pulse, which both have the same energy. We measured the dielectric spectrum of the pulsed cell suspension

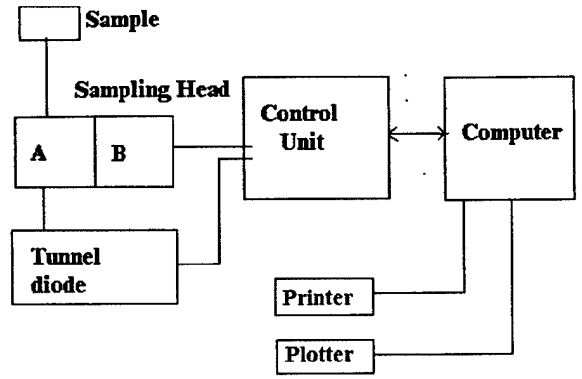

Figure 2: Circuit diagram of the TDDS system [7].

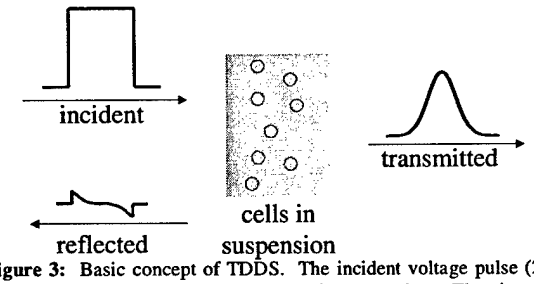

Figure 3: Basic concept of TDDS. The incident voltage pulse (200 mV, 5 us) is transmitted into the cells in suspension. The signal is partially reflected (into the sampling head and tunnel diode in Figure 2) and partially transmitted. This reflected signal provides the dielectric properties of the cell suspension, from which the dielectric properties of the individual cell can be obtained.

immediately after the pulse. For the 50 μs pulse, it took between ten and thirty seconds to conduct this initial measurement while it took about a minute for the 10 ns pulser because it is farther from the TDDS system.

After each measurement, we measured the cell viability by the trypan blue test. We then changed out the samples, ensuring that the control was measured periodically to serve as a baseline.

Results

After data acquisition, we conducted data analysis using the TDDS data analysis software. Whenever a highly conductive sample is placed in a small electrode gap, a surface layer of charge forms on the surface of the electrodes. This causes an artificial, parasitic effect called electrode polarization, which can mask the true electrical properties of the sample [7]. For the cylindrical sample cell used in these experiments, we used the single exponent method to model electrode polarization as an additional capacitance [7].

Once we compensated for electrode polarization, we determined the conductivity of the cell suspension. Figure 4 shows how the conductivity of the cell suspension changes as a function of time after the application of the 50 μs pulse. The conductivity of the cell suspension rose dramatically after pulse application. However, the conductivity *decreased* just as dramatically about forty minutes after the pulse. The conductivity returned to the average value of the control about eighty minutes after the pulse.

Electrically, one can consider conductivity as a measure of the ease with which ions traverse the individual membranes or, in this case, the entire suspension. Biologically, this means that when the conductivity of the cells rises, more ion transport takes place. Various means of transport exist through the cells, particularly in the cell membrane and the very porous nuclear membrane [1]. In particular, the initial increase in the conductivity could mean that either more ion channels are opened in the cell membrane or pores are formed in the nuclear membrane. Alternatively, it could mean that more Ca^{2+} or other ion channels have been opened and no change in pore formation has occurred. The rapid drop after forty minutes could be related to a delayed reaction that resulted in a closure of these open channels and other channels that were open in the control cells. Changes in cell shape and size can also impact the dielectric properties of the suspension. In particular, pulsed cells undergoing apoptosis have been observed to change shape and we are currently investigating whether pulsed HL-60 cells not undergoing apoptosis may exhibit similar behavior [5].

We then repeated these measurements for a 78.5 kV/cm, 10 ns pulse, which we would anticipate to affect the nuclear envelope more than the cell membrane [5]. Once again, we performed TDDS measurements of a control and a pulsed sample as a function of time after the pulse, as shown in Figure 5. Unlike the 50 μs pulse, the conductivity for the 10 ns pulse initially *decreased* before increasing above the average control value. This effect varied depending on how much time was required to transfer the sample from the pulser to the sample holder. In the cases where it took longer to measure the initial conductivity, we observed that the conductivity increased, similar to the effect seen for the longer pulse. Based on these results, we speculate that the short pulse may initially cause some ionic channels to close before reopening them and other channels as well. Once open, these channels remained open until about forty minutes after the pulse, as in the case of the longer pulse. Once again, the conductivity decreased substantially at this point, which may be due to the closure of ion channels. The conductivity then rose again but, unlike the 50 μs pulse, approaches a value *above* the average control value. This suggests that more ionic channels are opened and remain open for a long period of time after the pulse. The viability did not significantly change

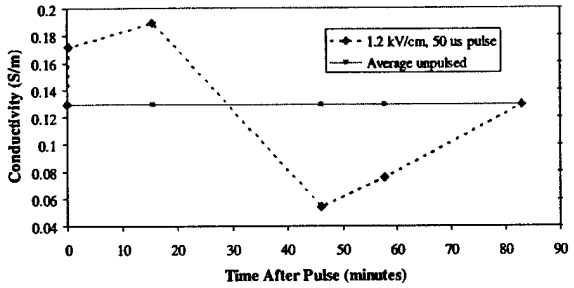

Figure 4: Conductivity of a 5% HL-60 cell suspension as a function of time after a 1.1 kV/cm, 50 μs pulse. The conductivity of the suspension rose dramatically after the pulse and fell rapidly forty minutes after the pulse. The conductivity returned to the average value of the control eighty minutes after the pulse.

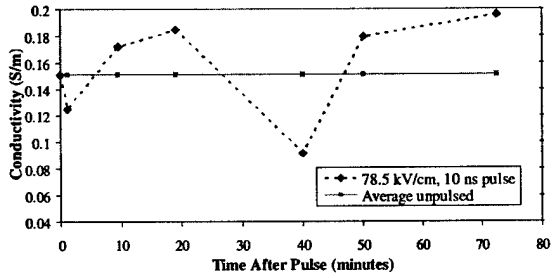

Figure 5: Conductivity of a 5% HL-60 cell suspension as a function of time after a 78.5 kV/cm, 10 ns pulse. Unlike the 50 μs pulse, the conductivity in this case initially *decreased* after the pulse before increasing a couple of minutes after the pulse. The conductivity dramatically declined forty minutes after the pulse, as in the 50 μs case, before increasing again. In this case, the conductivity remained higher than the average control value.

over the time of measurements. More experiments are required to determine whether this effect always occurs.

The behavior of the conductivity in the first minute or so after the pulse is significantly different for the microsecond and ultrashort pulses. The conductivity reaches a minimum about forty minutes after for both pulses. After this minimum the conductivity returns to the control value over a long timescale for the microsecond pulse; however, it rises above the control value for the ultrashort pulse.

Conclusion

By using TDDS, we have shown that applying either a 1.1 kV/cm, 50 μs pulse or a 78.5 kV/cm, 10 ns pulse alter the conductivity of HL-60 cell suspensions. We speculate that this is due to changes in the various ionic channels in the cell membranes and nuclear envelopes. Particularly interesting is the rapid drop in conductivity forty minutes after the application of both types of pulses.

Our next step is to place this data into the two shell model and determine how these pulses effect the dielectric properties of individual HL-60 cells and normal human peripheral blood lymphocytes. Understanding the different effects of pulsed power these cells might lead to future applications of pulsed power in cancer treatment.

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