BIOELECTRIC STUDIES WITH SUBNANOSECOND PULSED ELECTRIC FIELDS

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Abstract

Nanosecond electrical pulses have been successfully used to treat melanoma tumors by using needle arrays as pulse delivery systems. Reducing the pulse duration of intense electric field pulses from nanoseconds into the subnanosecond range, and using a prolate-spheroidal reflector as part of a picosecond Impulse Radiating Antenna (IRA), allows us to focus the electromagnetic waves into biological tissue with reasonable spatial resolution. In order to achieve a spatial resolution on the order of one centimeter, pulses with duration on the order of 100 picoseconds are required. Based on the nanosecond pulse generator, a pulse generator was developed which allows us to generate 150 picosecond-long pulses. The voltage amplitude (in an improved version) reaches values of up to 120kV. Modeling results indicate that with this pulse generator as part of an IRA, electric fields on the order of 100 kV/cm can be generated in tissue close to the body surface. In order to explore the biological effects of these ultrashort, high electric fields, a coaxial exposure chamber has been designed which is integrated into the pulse delivery system in such a way that an almost uniform electric field (based on modeling using MAGIC) can be expected. The chamber is placed in a water bath, which allows us to vary the ambient temperature from room temperature (20 C) to a physiologically relevant range (37 C to 41 C). Experiments where platelets were exposed to 150 picosecond long pulses with an electric field of 150 kV/cm indicate a pulse number dependent uptake of calcium. The experiments were performed at a temperature of 37 C. A possible synergistic effect was observed when melanoma cells were pulsed at elevated temperatures.

I. INTRODUCTION

Skin tumors in mice have been successfully treated with 300 ns pulsed electric fields with voltage amplitudes up to 10 kV [1,2]. These pulses were delivered to the tumor with needle electrodes, or with plate electrodes surrounding the tumor. In order to be able to treat tumors beneath the skin, a non-invasive technique based on a prolate-spheroidal irradiating reflecting antenna (IRA) is being explored. In this antenna, an impulse is radiated from the first focal point, and the electric field is focused at the second focal point, as schematically shown in Figure 1. The risetime of the impulse function determines the pulse width of the signal at the focal point, as well as the spatial resolution. In order to achieve a spatial resolution of about 1 cm, a risetime of 100 ps is needed, which generates a pulse at the focal point with a pulse width of 100 ps [3,4,5]. A pulse generator has been built to study the effects of these pulses on cells in vitro [8]. In this paper,

Figure 1: An impulse signal is fed into the first focal point, and the electric field is focused at the second focal point [3,4,5].

the pulse generator, and the design of the coaxial exposure chamber will be covered. The voltage diagnostics will be discussed, followed by biological experimental results.

II. PULSE GENERATOR

The schematic of the pulse generator is shown in Figure 2. A four stage Marx Bank discharges through a peaking switch into a 50 Ω cable terminated by a 50 Ω load.

Figure 2: Schematic diagram of the 150 ps pulse generator [8].

A tailcut switch is placed in front of the load to cut off the tail part of the pulse. All six switches of the system are spark gap switches, in which the four Marx Bank switches are pressurized up to 50 psi with nitrogen, while the peaking switch and tailcut switch are at atmospheric pressure in air. The electrodes in the Marx Bank have a semi-spherical shape. The first set of electrodes are placed 1.5 mm apart while the remaining three are placed 2.5 mm apart. This is to insure self breakdown at the first electrode at all times.

The geometry of the peaking switch is the same as the Marx Bank switches with the only difference in the radius (3.5 mm) and the gap distance between the spark gaps (1.4 mm). The peaking switch causes a decrease in the risetime of the pulse from the Marx Bank, as well as doubling the voltage. The peaking switch has an inductance and a stray capacitance (2 orders of magnitude smaller than the capacitance from the Marx Bank) which results in a resonance charging circuit with

the Marx Bank. In order to get voltage doubling, the inductance of the switch, L , needs to be

$$L = \frac{t_r \cdot Z}{2} \quad (1)$$

where Z the impedance of the system (50Ω), and t_r is the risetime of the pulse (100 ps). The inductance of the switch can be varied by changing the gap distance between the electrodes, or by varying the radius of the plasma channel of the spark (by adjusting the pressure).

The tailcut switch is placed within the cable 8 cm in front of the load and causes the inner conductor to break down to the outer conductor of the cable. A 50Ω cable with polyethylene insulator (RG217/U) is connected to a 1 cm long 50Ω cable with air insulator and back to the RG217/U cable as shown in Figure 3.

Figure 3: A 1 cm long 50Ω air insulated cable is inserted into a RG217/U cable to create the tailcut switch.

This setup allows the signal to travel with negligible disturbance through the tailcut switch. This arrangement decreases the jitter of pulses by having the breakdown occur strictly between the two electrodes rather than the wall of the hole of the insulator (surface breakdown) as described in reference 8.

III COAXIAL CHAMBER

The exposure chamber must be designed to generate a uniform electric field across the cell sample as well as being matched to the 50Ω system. If the chamber is not matched, the pulse will be reflected back and oscillate between the load and the shorted tailcut switch.

The pulse generator is capable of delivering a 150 ps pulse with an amplitude ranging from 80 kV to 120 kV. In order to generate an electric field ranging from 100 kV/cm to 150 kV/cm, the length of the load (for a matched system) was set to 8 mm. To insure a uniform electric field, the radius of the sample must be less than the risetime of the pulse in the sample. The fundamental wavelength of the pulse with water in the chamber is given as:

$$\lambda = \frac{c}{\sqrt{k}} \cdot t_r = \frac{\left(3 \cdot 10^8 \frac{\text{m}}{\text{s}}\right)}{\sqrt{81}} \cdot 100\text{ps} = 3.3\text{mm} \quad (2)$$

with c being the speed of light, and k the dielectric constant of water.

There also needs to be sufficient volume to perform biological measurements, therefore the radius of the sample should be as large as possible. It was chosen to be 2.3 mm. The sample is placed at the end of the inner electrode (shown

in Figure 4). The resistance of the sample (assuming water) was calculated to be 448Ω . In order to be matched, a resistor (shown in black) needed to be placed in parallel with the sample (shown in dark gray).

Figure 4: Diagram of the coaxial exposure chamber. The resistor is shown in black, while the sample surrounding the resistor is shown in dark gray. The insulator is polyethylene.

The radius of this resistor is 1.2 mm, which increases the resistance of the sample to 61Ω due to its reduced cross sectional area. In order to be matched to a 50Ω system, the value of resistor was calculated to be 55Ω . The placement of the resistor limited the sample volume to 100 μL , which is still sufficient for biological studies.

The outer radius of the sample (2.3 mm) is larger than the commercially available radius of the cable (RG217-U) which has a value of 1.34 mm. A coaxial waveguide was therefore designed and built in order to deliver the pulse to the sample with minimum reflection (Figure 4). The radius of the outer conductor was designed to keep a 50Ω impedance throughout the waveguide structure. In order to determine the distribution of the electric field within the sample, MAGIC software was used [10]. A voltage pulse of 150 ps with an amplitude of 100 kV was used in the simulation. The electric field was recorded from the inner conductor to the outer conductor (0 mm – 8 mm) and from the inner radius to the outer radius (1.2 mm – 2.3 mm). The results are shown in Figure 5.

Figure 5: Electric field distribution in the exposure chamber. The x axis represents the axis from the inner radius to the outer radius of the sample and the y axis represents the distance from the bottom to the top of the sample.

Given the results by MAGIC, we can conclude the electric field will deviate by no more than 2.5% of the average value.

IV. DIAGNOSTICS

The voltage is measured with a capacitive voltage divider 6 cm in front of the load within the cable as shown in Figure 6.

Figure 6: The capacitive divider is placed 6 cm in front of the load and 2 cm behind the tailcut switch.

The geometry of the voltage probe is shown in Figure 7. The time constant of the probe ($R_p C_1$) must be very large compared to the pulse width (150 ps), where C_1 is the series capacitance of C_1 and C_2 , in order to obtain an accurate measurement. To achieve this, the resistive value of R_p was chosen to be 2.3 k Ω , which gives a time constant of 8.3 ns.

Figure 7: C_1 is the capacitance between the inner conductor of the cable and the thin metal sheet, and C_2 is the capacitance between the metal sheet and outer conductor of the cable.

The voltage divider ratio of the capacitive divider ($V_{\text{signal}}/V_{\text{probe}}$) was determined by calculating the capacitances, C_1 and C_2 . C_1 is the capacitance between the inner conductor and the thin metal sheet, and C_2 is the capacitance between the outer conductor and the metal sheet. They are 0.1 pF and 6 pF respectively. The resistor of the probe (R_p) connected to the 50 Ω termination resistor of the oscilloscope (4 GHz) forms a voltage divider. The voltage divider ratio of the probe was then calculated using Equation 3.

$$\frac{V_{\text{signal}}}{V_{\text{probe}}} = \frac{C_1 + C_2}{C_1} \cdot \frac{R_p + 50\Omega}{50\Omega} = 2867 \quad (3)$$

One calibration technique involved using a low voltage (< 1 kV) FID pulse generator. First, the FID is terminated into the oscilloscope through two 20 dB attenuators, which allows the voltage to be measured directly from the pulse generator. Second, the pulse generator was terminated using a 50 Ω load, and the voltage was measured by means of the capacitive divider. The voltage divider ratio was calculated by dividing the peak value of the voltage measured directly from the oscilloscope with the peak value of the voltage measured with the capacitive voltage divider. The ratio (including the

resistors) was found to be 3223, which is close to the theoretical value. The two pulses are compared in Figure 8, with each trace being an average of 1,000 pulses.

Figure 8: Comparison of the pulse from the FID pulse generator measured from the oscilloscope compared to the pulsed measured from the capacitive divider.

The probe attaches to the oscilloscope by a 3 meter 50 Ω cable (outer diameter: 2.1 mm), and a 20 dB, SMA attenuator (PE7045-20). The frequency response of the cable and the attenuator was measured using an 8753E 3 GHz network analyzer. The change in the impedance of the system as the frequency was increased was found to be negligible. A typical waveform of the pulse with the coaxial chamber is shown in Figure 9.

Figure 9: 150 ps, 120 kV pulse across the exposure chamber.

V. EXPERIMENTS

Experiments were performed on B16-mouse melanoma cells to determine the lethality of the pulses, and on human platelet cells to determine the degree of platelet activation. Platelet clotting is related to the amount of calcium release by the platelet cells [9]. The pulsing conditions ranged from 1 pulse to 10,000 pulses and the electric field ranged from 100 kV/cm to 150 kV/cm.

For the platelet cells, the temperature was kept at 37°C by using a water bath surrounding the cell chamber. The platelets were exposed to 400 pulses with an electric field of 150 kV/cm. After pulsing, cells were studied to see if they released a higher amount of calcium than normal since calcium release is a precursor to clotting. Although no

clotting was observed, there was a noticeable change in the amount of calcium release, as is shown in Figure 10.

Figure 10: Calcium response of platelet cells due to pulsing.

Time 0 represents when the cells were placed in the fluorometer for testing. Cells that were not pulsed had the same variations in calcium, but with a slope of zero while the cells that were pulsed had a negative slope. If the slope is extrapolated to the time of the last pulse, there is a higher release in calcium which suggests the pulses are stimulating calcium release.

The B16 cells, when pulsed at room temperature (22°C), were not affected by the pulses. The number of pulses varied from 500 to 1,000 with an electric field of 100 kV/cm. The temperature of the cells was then raised above 30°C and there was no affect due to the increase in temperature alone. However, when the temperature of the cells was raised above 30°C, and then the cells were exposed to pulses, there was a synergistic effect between the pulses and temperature, as shown in Figure 11 (1,000 pulses, 100 kV/cm). When the cells were pulsed at increased temperature, there was a 5% difference from the control.

Figure 11: Trypan blue exclusion of B16 melanoma cells due to pulsing and heating.

VI. CONCLUSION

In order to determine the bioelectric effects of ultrashort pulses as used in IRA's, we have developed a pulse generator

which creates pulses with pulse widths of 150 ps, rise time of 100 ps, and voltage amplitudes up to 120 kV. The pulse generator uses a peaking switch and a tailcut switch to decrease the risetime and cut off the tail part of a 4 stage Marx Bank pulse, respectively. A coaxial exposure chamber was designed to deliver a semi-uniform electric field to the sample which deviates from the average value by less than 2.5%. It was also designed to be matched to the system, as well as have sufficient sample volume for biological studies. The voltage diagnostic is based on a capacitive voltage divider placed within the cable 6 cm in front of the load. The platelet cells released calcium when exposed to 400 pulses with an electric field of 150 kV/cm. Clotting of the platelet cells could possibly be observed with an increase in the pulse number, or the electric field. There was no effect on the melanoma cells with pulses alone. However, with raising the temperature of the melanoma cells to physiological temperatures, there was a synergistic effect. In order to utilize this effect, a system is being developed which allows simultaneous heating and pulsing.

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VII. REFERENCES

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