Nanopulse Generators: Their Design and Application to Cancer Therapy Studies

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Abstract

Effective nanopulse generators have become critical in recent decades concerning the study of subcellular affects in response to nanosecond pulsed electric fields. It has been observed that nanosecond duration electric pulses can target intracellular organelles, ultimately leading to cell apoptosis, suggesting the possibility of a new, minimally invasive, low risk cancer therapy methodology. The standard topology for developing a medical nanopulser is the Blumlein "transmission line" approach. This approach relies on the nearly infinitesimal, yet finite amount of time required for an electromagnetic field to propagate down a short transmission line. Prior to design, requirements and constraints must be defined that are determined by the specific applications and experiments that the nanopulser will be used for. Special effort must be put into nanopulser design to prevent undesirable reflections and oscillations at the load. Critical design objectives common to most nanopulse generators include choosing effective switching elements that facilitate a minimal rise time, configuring the load electrodes to be compatible with experimental setups, and enabling a wide degree of versatility and adjustability concerning pulse parameters.

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Introduction

Pulsed power systems have found many applications and areas of research in recent decades. Nanopulse generators, generators that can produce pulses with nanosecond-duration time length, have recently been used in the biological and medical field to study subcellular effects caused by "nanopulses." More specifically, experiments have been conducted to test whether nanopulses can consistently produce induced cell apoptosis.

Electrical therapy has been used in the past and presently today as a cancer treatment methodology, and nanopulse-based treatments have become a subject of interest among medical researchers. Equivalent-circuit electrical models have been developed to represent the cell, and theories have been developed in attempt to explain how nanopulses affect the cell interior. Prior to nanopulse experimentation, however, competent nanopulse generators must be developed and designed that meet experimental requirements and objectives.

Nanopulse generators or "nanopulsers" tend to present numerous challenges during design due to the high-voltage, ultrashort nature of the pulses they are expected to produce. Various topologies such as the Blumlein circuit scheme have been referenced in order to generate pulses at this small scale. Critical design choices must be made when selecting appropriate switching elements and placing appropriate electrode configurations on the generator. Versatility of generators and the degree of adjustability for pulse parameters can also play a crucial role in the overall effectiveness and quality of the generator.

Studies and research in the effects of nanosecond pulsed electric fields (nsPEFs) have lead to this emphasis on designing effective "nanopulse" generators. Nanopulse generators require significant attention and consideration during their design, and must be tailored to the needs of each particular nsPEF experiment.

Background on nsPEFs and Nanopulse Generators

Electrical Therapy in Cancer Treatment

For many decades now, various medical procedures and modalities rely on the use of electricity and electromagnetic fields to facilitate particular treatments. In particular, new treatments for cancer using electromagnetic fields have led to treatments that are "non-surgical" and "minimally invasive" [1]. Many techniques have been used or studied to date, ranging from but not limited to radiofrequency ablation, microwave hyperthermia, focused ultrasounds, electrochemical treatments, electrochemotherapy, irreversible electroporation, and of course, nanosecond pulsed electric fields (nsPEF) [1].

Many of these electricity-based treatments have pros and cons that affect the frequency of their use in the clinic and current research efforts. For example, many of these treatments result in induced heating of the cancerous tissue, specifically the techniques that don't utilize electroporation. For example, radiofrequency ablation relies on electromagnetic waves that will induce a current in cancerous tissue that causes resistive heating, ultimately leading to coagulative necrosis, or "the death of…cells…in an organ or tissue, caused by disease, injury, or interference with the blood supply" [2]. This heating induces hyperthermia in the applied cells, destroying both cancerous and normal tissue positioned between the electrode and grounding pad [1].

One of the more selective and non-invasive cancer treatments is electrochemotherapy, a treatment that "associates electricity with anticancer drugs" [1]. The procedure begins with an injection of non-permeable chemicals with high intracellular cytotoxicity into the tumor. Electrical pulses are then applied to the body of tumorous cells, causing cell permeabilization, the allowing of non-permeable molecules to pass through the cellular membrane. Therefore, only the cells exposed to the electrical pulses are exposed to the cytotoxic chemicals which then eliminate the cancer cells. This process of permeabilization is known as cell electroporation, "the permeabilization of the cell membrane induced by exposure to short and intense electric pulses" [1]. There are many advantages to this method of cancer treatment: electrochemotherapy is easy, effective, safe, cheap, highly selective, and causes minimal side effects [1].

The potential use of nanosecond pulsed electric fields provides the hope of a purely electrical cancer therapy without any need for drugs, contrary to electrochemotherapy. Additionally, nsPEFs target and eliminate cancer cells without hyperthermia, or local pH changes that can negatively affect surrounding healthy tissue. The overarching goal of investigating treatments such as these, is to discover methods of treatment that are non-invasive, involve limited surgery, reduce pain, reduce scarring, reduce patient mortality, and are cost-effective and safe [1].

Theory of nsPEF Effects on Cells

When applying electrical signals and pulses to a group of cells, each individual cell can be thought of as a "conductive body" that consists of the cell's cytoplasm, with a "surrounding dielectric" that consists of the surface membrane [3]. Electrical equivalent circuit models can be developed to adequately represent the electrical properties of

different kinds of cells, for example, the below model in figure 1, created by [3], represents the electrical parameters of a cell suspension.

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Figure 1: Electrical Model of a Spherical Cell

Given this model, the time needed to charge the cell is dependent on the constitutive parameters of the cell and medium. Assuming an ideal dielectric surface membrane (no leakage), the charging time constant can be found by — , where is the capacitance of the surface membrane per unit area, is the cell diameter, is the resistivity of the cytoplasm, and is the resistivity of the medium that the cell is suspended in [3]. According to [3], such models as these are based on "relatively crude assumptions."

Based on the above mathematical model, — , it can be seen that the variable, , is directly proportional with the time constant, , meaning that the greater the membrane diameter, the greater the time constant. Since the time constant essentially represents the length of time required for the membrane to be completely charged, it stands to reason that if two cellular structures were exposed to the same

electric field intensity, but had different membrane diameters, then the cellular structure with the lesser membrane diameter should ideally fully charge first.

Given this conclusion, it can be hypothesized that because a cell has an exterior membrane, and a smaller, interior nuclear membrane (and other small intracellular membranes), the nuclear membrane will become fully charged before the exterior membrane is fully charged [4]. If the exterior membrane reaches a point where it is fully charged, an electromagnetic shielding effect is produced, essentially "shielding" the intracellular organelles and membranes from the electric field [4]. According to [5], this is caused by ions in the cell responding to the electric field by moving with or against the direction of the field. The redistribution of charges will eventually establish an equal and opposite field, relative to the exterior field, producing a net electric field of 0 V/m within the cell interior.

It can be concluded from the above premises that by applying multiple pulses that are shorter than the charging time constant of outer membrane, but equal to or longer than the time constant of the intracellular membranes, permeabilization should repeatedly be induced in the intracellular membranes, while the outer membrane essentially remains unaffected. The intracellular membrane will be repeatedly and fully charged and discharged, while the exterior membrane will only be marginally, negligibly charged and discharged. This permeabilization of intracellular membranes presents a new means of inducing apoptosis in cancer cells. It is important to note that sufficient time must be given between nanopulses to allow the intracellular membranes to completely discharge.

Since a pulse meeting the earlier described criteria will typically be in the nanosecond range, and the energy of a pulse can be represented as $W = \frac{V^2}{R_L}\tau$, it is intuitive that the energy (W) dispensed with each pulse will remain small since the pulse length (τ) is directly proportional with it, therefore causing minimal heat transfer, and reducing any chance of induced hyperthermia in the cells. This is an important feature of nanopulses, since a nanopulse-based cancer treatment seeks to avoid standard toxin and hyperthermia-based treatments.

While there is certainly evidence for all of the above premises to be true, there still remains many questions concerning the mechanism of action that nanopulses truly affect on a targeted group of cells [1]. This is why it is especially important that effective and reliable nsPEF generators are designed and used in nanopulse-oriented experiments.

Why Nanopulse Generators are needed and Present Challenges

The importance of effective nanopulse generators is emphasized by [1]: "the nsPEF experiments were made possible because of major improvements in the design of nanopulsers. Their fabrication is highly complicated due to the high voltages and ultrashort durations needed. The main challenge is to deliver a repeatable pulse with a minimum of oscillations and reflections in the waveform." In short, the effects that high-voltage, nanosecond-duration electric pulses have on cells cannot be observed unless there are generators that can create such pulses.

It is essential that the duration of the pulses are guaranteed to be in the sub-microsecond range to avoid "ordinary electroporation effects dominant at long pulses" [6]. It can be generalized that pulses greater than 1 μs will fully charge the outer

membrane for an average cell, while pulses less than 1 μs will generally affect organelles in the cell interior [6]. Ranging from study to study, there is a need for these pulse generators "to meet specific biomedical and biotechnological research requirements" [7].

Because most research efforts require variable pulse parameters as a requirement within an experiment, it means that pulse generators need to be developed with a degree of versatility. Given many of the current topologies used in nanopulse generator design, versatility can be one of the more challenging facets of design: "Generators that can produce powerful electrical pulses with adjustable duration, amplitude, and shape are convenient but still unusual" [8]. By introducing this adjustability to a nanopulse generator, medical researchers can effectively test the effects of certain pulse parameters on cellular response.

In addition to versatility, applying a high-voltage electrical pulse to a biological load often creates challenges. While a pulse generator may exhibit ideal pulse properties across a purely resistive test load, there may be unexpected loading and impedance mismatch effects when a pulse is applied to a biological load. Even when the generator effectively generates short, high voltage pulses, with significantly short rise and fall times, it remains a significant challenge to apply a pulse to a biological target with exactly the pulse characteristics desired [9]. Given these challenges in designing a nanopulse generator, it is important to fully define the experiments they will be used for, and how the electrical pulses will be applied.

Experiment and Design Requirements

Experimental Setups and Considerations

An essential step that must be made, prior to formulating the design requirements for a nanopulser design, is to fully define the experiment or study in which the nanopulser will be used. Cells have been primarily exposed to electrical pulses in one of three ways: 1) under exposure in an electroporation cuvette [9], 2) within an electrode gap on a microscope slide [3], and 3) within an electrode gap on a live specimen's tissue [5]. Numbers 1 and 2 would be denoted as an "in vitro" experiment, while number 3 would be denoted as an "in vivo" experiment. In vitro experiments tend to be convenient for studying interactions between cells and electrical fields on a theoretical basis without accounting for the complexity of a biological tissue [10], while in vivo experiments tend to offer more empirical, practical results.

An electroporation cuvette is basically a small, box-shaped container with two parallel plate electrodes that can sustain an electrical field across any biological media or cell suspension that it contains [10]. Electroporation cuvettes are cheap, simple, and accessible, but certainly have some drawbacks. As will be discussed further along in the paper, electroporation cuvettes have a tendency to cause impedance matching and reflection concerns during pulse application [9]. Also, it is difficult to observe real-time cellular response to nanopulses with a cuvette, as it is difficult to integrate it into a microscope-based setup.

Microscope systems tend to offer numerous advantages and conveniences as it pertains to nanopulser design. For example, due to the compact nature of a microscope arrangement, it often results in lesser electrode gap distances, usually 100 μm or less

[11]. Because of this small gap, smaller voltages are needed to produce the same electric field intensities, assuming the electric field can be modeled by – for a uniform field. Because lower voltages are needed, simpler switches can be used to activate the nanopulse, for example, a MOSFET transistor switch [11]. Also with microscope systems, it is possible to incorporate a camera into the design to capture continuous images of cellular response. For example, [3] introduced a low-light, computer-controlled CCD camera to their experimental configuration to capture a wide range of pulse responses both before and after pulse application; the whole setup can be viewed below in figure 2.

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Figure 2: Experimental Setup for Microscope System

The last experimental setup involves direct application of nsPEFs to a living organism's tissue, or an "in vivo" experiment. No clinical trials have currently been performed with nsPEF application, and in general, not many in vivo studies have been conducted on tumors [1]. Several experiments, however, have involved in vivo studies with mice that have shown positive results and cancer remission [1], [5]. With in vivo

experimentation, it is important to consider the impedance mismatch effects, and the electrode configuration when directly applying the pulses to a large portion of tissue.

Some additional concerns to consider when preparing a nsPEF experiment include determining what kind of pulse characteristics are required, and which pulse characteristics should be variable. In order to prevent a temperature increase of cells during pulse application, it is often beneficial to maintain a constant energy level of each pulse. With this in consideration, based on the earlier described formula, $W = \frac{V^2}{R_L}\tau$, if pulse amplitude or time is a variable, it is important that both amplitude and time will change inversely in order to maintain a constant energy level and comparable effects [12].

Design Requirements and Specifications

In any design, one of the preliminary steps is to draft basic design requirements and constraints, which, in the case of a nanopulser, will be based upon the objectives and functions expected of the generator in relation to the specific experimental setup. These design requirements and constraints will essentially create a design space that defines the possibilities of the design. For example, some setups require that the pulse amplitude and duration must be inversely related and variable to produce a constant amount of energy with each varying pulse [4]. This would be one of the design requirements and ultimately influence the design space.

The design requirements can be driven by, but not limited to, such factors as the load impedance, physical form of the load (i.e. electroporation cuvette or microscope slide), pulse amplitude, pulse duration, minimum rise time, and the degree of adjustability

of the pulse parameters; the values for these items will ultimately direct the nanopulse generator's topology [13]. Put another way, "the design specifications for the pulse generator are calculated based on the desired electric fields and the geometry of the electrode micro-chamber containing the cells under study" [14]. An application by [7] dictated that the particular design be compact and feature a wide range of pulse properties such as adjustable amplitude, rise time, and pulse duration.

If the threshold field amplitude needed for intracellular effects is known, as well as the electrode configuration needed, the applied voltage can be determined [12]. Specifications by [13] stipulate that the generator be able to conveniently interface sterile load chambers, offer variability of pulse parameters as experimental variables, operate within correct electrical impedance matching and pulse shape constraints, and remain compatible with observational techniques used to access cellular response. All of the above cases illustrate the nature between design requirements and experimental goals. In summary, the pulse amplitude, duration, rise time, pulse shape, adjustability and versatility, load characteristics, and application environment will all shape the requirements that define the design and the constraints that limit the design space.

Designing a Nanopulse Generator

Nanopulse Generation Topologies and Methods

Since nanopulse generator design first became a topic of interest, there has been multiple approaches and topologies created to meet design requirements. Various topologies include but are not limited to the Blumlein transmission line approach, Marx Bank concept, Pichugin principle, fast recovery diode scheme, and magnetic pulse compression generators [6], [7], [8]. The two that will be focused upon primarily will be

the Blumlein and Marx bank schemes, each finding applications in cellular response studies. First to be explored will be the Blumlein scheme, perhaps the most common among nanopulse applications.

Blumlein circuits as they apply to nanopulse generation can be referred to as a "transmission line approach" since they essentially use a voltage step propagating down a transmission line to obtain an exceptionally short pulse duration. Two examples of typical line type Blumlein circuits can be seen below in figure 3 [4], [15].

Figure 3: A Blumlein Circuit

As is evidenced from figure 3, one of the ends of the conductors is charged to an initial high voltage, , and the other end is grounded, while both are connected to the load, . When the closing switch is closed, a pulse is generated across the load, with a duration lasting the needed length of time for the voltage step to propagate down the transmission line. The speed of electromagnetic propagation down the transmission line is comparable to the speed of light, which is approximately m/s. Specifically, the pulse duration can be defined by the transmission line's length and the dielectric's constitutive parameters, mathematically modeled by , where is the length of the whole transmission line, and and are respectively the magnetic permeability and electric permittivity of the dielectric. The characteristic impedance of the strip lines can

be modeled by – – where is the separation between conductors, is the width of the strip line, and and again correspond to the dielectric [4].

Based on the formula for pulse duration, —, it is apparent that in order to modify the length of a nanopulse, the physical length of the actual transmission line must be modified. In an experiment where a variable pulse duration is required, it will be important to develop a design that facilitates simple and easy transmission line "swapping."

One of the common concerns with the Blumlein approach to circuit design is what is known as electromagnetic reflections. If the load is not matched with the characteristic impedance of the transmission lines, a series of pulses with gradually decreasing amplitudes will appear at the load. If the load is matched properly, the applied voltage across the load should ideally equal that of the charging voltage. If this effect is present, the boundary reflection can always be minimized by adding an appropriate parallel resistor with the load, assuming the load impedance is greater than the transmission line characteristic impedance. Figure 4 provides a visual representation of such reflection and impedance mismatching effects [4].

Figure 4: Impedance Mismatch & Reflection Effects

Assuming there are minimal impedance matching and reflection concerns, output pulses from Blumlein circuits generally appear as they do below in figure 5. Each pulse represents a negative polarity, high voltage, nanosecond duration pulse, with the first representing a lesser voltage pulse applied to a microscope slide sample, and the second representing a higher voltage pulse applied to a larger volume such as a suspension in an electroporation cuvette [4].

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Figure 5: Blumlein Circuit Nanopulses

Transmission line generators like the Blumlein generator are advantageous because they are well suited for high voltages and fast switching; they are based on a simple, consistent concept and can sustain fields on the order of MV/m amplitude [13]. While figure 3 presented two strip line models of Blumlein generators, they also are commonly constructed with coaxial cables, which tend to have higher voltage-handling capabilities and safer operation [8]. A simple representation of the coaxial cable model by [13] can be seen in figure 6. An additional benefit that Blumlein circuits provide is that the delivered pulse is equal to the amplitude of the charging voltage, contrary to other systems that will only deliver half of the charging voltage [13].

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Figure 6: Blumlein Circuit with Coaxial Cable Transmission Lines

Contrary to the Blumlein topology, the Marx bank topology actually creates a high voltage pulse with a greater voltage than the charging voltage. The basic principle of the Marx bank, is to charge multiple capacitors in parallel, then nearly instantaneously discharge all the capacitors in series synchronously. Switching devices are purposefully placed near each capacitive element to cause an action-reaction "avalanche" effect that very rapidly transitions each capacitor's configuration from parallel to series [7].

Several BJT avalanche transistors were used by [14] for the switching elements, and the circuit was described as "a series chain of avalanche transistors and capacitors configured as a tapered transmission line from high voltage to ground," being interpreted as another transmission line based approach. The nanopulse generator by [14] in its entirety can be observed in figure 7.

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Figure 7: Avalanche Transistor Nanopulse Generator

In both avalanche transistor circuits by [7] and [14], the BJT transistor switching element will rapidly switch from a high voltage, low current state to a low voltage, high current state. Each BJT from one end of the circuit to the other will breakdown in successive stages, ultimately summing all of the capacitive elements [7]. The breakdown at the first transistor is triggered by a signal from a trigger source circuit, forcing the base terminal to be shorted to the emitter; each subsequent transistor breakdown is caused from the previous stage. This successive breakdown is the reasoning behind the naming of the "avalanche effect" [14].

The circuit by [14] is "tapered" in the sense that each successive stage has a higher impedance than the previous one (notice the decreasing capacitance with each stage), ultimately causing each stage to "avalanche" faster than the previous one. It is important that the tapering have smooth transitions in order to avoid significant reflections caused by large impedance jumps [14]. In the end, this approach can result in rise times as short as 100 picoseconds [14], a parameter that is commonly desired to be as short as possible.

Another important objective of the avalanche transistor approach is that a uniform DC bias be applied over every stage; failure to implement this could lead to an undesired trigger to be supplied. Symmetric voltage division can be achieved through using high-voltage zener diodes as seen earlier in figure 7 [14]. Below in figure 8 is a measured nanopulse produced by the design by [14], with a pulse width of 1.3 ns and a rise time of only 0.8 ns. It is beneficial to note the difference between this pulse and the pulses in figure 5: the Blumlein pulses tend to have a more rectangular shape, while Marx generator pulses tend to have a more "sharp," non-rectangular pulse, which are both

important features to note when deciding which configuration will more suitable for a given application.

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Figure 8: Avalanche Transistor Nanopulse

Electrical Switch Considerations

An electrical switch of some shape or form is almost always a part of a nanopulse generator, and very often a critical element in facilitating short pulse durations and rise times. There is a very large pool of potential switch elements that could be used as a nanopulser switch, ranging from but not limited to optoelectronic switches, power semiconductors, pressurized spark gaps, thyristors, diode opening switches, thyratrons, silicon-controlled rectifiers, or solid-state switches like MOSFET and BJT transistors [7], [8].

One of the common means of minimizing rise time is through using the spark gap switch. A spark gap consists of two electrodes that are separated by a gas volume, and when a particular threshold voltage level is exceeded, the gas insulation between the electrodes fails and a plasma channel forms, shorting the gap and activating the switch. The particular threshold voltage is determined both by the electrode gap distance and the

gas pressure. Generally, very short distance, high pressure spark gaps are preferred to induce a very short rise time. Charging systems can be easily connected with the spark gap to implement continuous charging and discharging cycles for repetitive pulse application. Spark gaps can provide the nanopulser with continuous pulses with rise times on the order of 1 nanosecond [4].

Though the spark gap switches have advantages, there are also disadvantages. Spark gaps tend to have a large size, high maintenance requirements when compared to other switches, a short lifetime, erratic behavior, and high jitter; they are particularly inefficient when pulse trains with a low frequency are required [6], [7]. It is further described by [7] that nanopulse generator designers have recently been shifting from sparks gap and vacuum devices to solid-state switches. One of the more common solid-state switches used in nanopulser design are MOSFET transistors.

MOSFET switches can create fast switch times with rise times as short as approximately 3 nanoseconds, a sufficient amount of time for most nanopulse experiments [4]. One disadvantage, however, of a MOSFET switch, is that it can only sustain a maximum voltage of about 1 kV, meaning that in order to pass a high electric field, the electrode gap distance must be very small, on the order of about 100 μm, which ultimately means MOSFET switches can only be applicable to generators used with miniscule, microscope-based configurations [4]. Figure 9 by [12] shows two Blumlein circuits, the top with a MOSFET switching element, and the bottom with a spark gap switching element connected to ground.

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Figure 9: MOSFET Switch (top) vs. Spark Gap Switch (bottom)

Another solid-state switch that can be used is the BJT transistor, used in avalanche transistor schemes [7], [14]. It is defended by [7] that "BJTs are well-suited for nanopulse switching applications," having a high transconductance and non-linearity resulting in low sensitivity to parasitics, high current density rating, long lifetime, stable operation, low jitter, and low inductance which is necessary for a fast rise time. In addition to BJTs, an optoelectronic switch called "laser triggered photoconductive semiconductor switches (PCSS)" provide another means of fast switching, and can be triggered by a simple laser configuration [9]. PCSS switches can operate in two differing modes depending on the needed configuration, linear or avalanche mode. Linear mode requires high optical energy for switching, but facilitates minimal jitter and high frequency rates, while avalanche mode requires less energy but reduces the device's lifetime and can cause jitter. Ultimately, as has been emphasized, the experiment and

design objectives and constraints will ultimately influence what the most optimal switch will be for a design.

Electrode Configuration

Electrode configuration will primarily be determined by what cellular environment the nanopulses will be applied to. For experiments involving in vitro exposure to a large group of cells, an electroporation cuvette is an ideal suspension environment to apply nsPEFs to. Commercial cuvettes will often have a coaxial cable connection point, leading to each electrode within the cuvette that will surround the cell suspension. With cuvette cell suspensions, there is likely to be reflection, with generator pulses and reflection pulses interfering with one another across the line and cuvette [10]. It is stated by [9] that even with short rise/fall times and high voltage pulses, it can still be very "challenging to effectively inject such [a] kind of signal down to [a] biological target using [an] electrode cuvette."

Custom electrodes will have to be integrated into microscope, real-time observation experiments. The "microreactor" developed by [4] uses simple microscope slide mountable, stainless steel, 100 μm thick electrodes. The electrodes are simply positioned around the microscope slide, and an electric field is generated across the cells under study. It is advantageous to utilize microscope slide electrodes when it is important to provide real-time imaging of cellular responses and morphological changes [13]. Similar to cuvettes, microscope slide electrodes remain vulnerable to impedance matching and reflection concerns. Particularly challenging with this configuration is enabling rapid load replacement when multiple cell samples need to be tested [13].

Two differing styles of electrodes can generally be utilized in in vivo experiments: 5-needle and parallel plate electrodes. The 5-needle array electrode used by [5] to treat melanoma tumors used five 30-gauge hypodermic needles extending from a Teflon base. Four needles are arranged in a square fashion, each forming the cathode, and a fifth needle is placed in the center of the other four, forming the anode. The parallel plate electrodes used by [5] were made from stainless steel, with each plate coated in a layer of conductive agar to separate skin from the actual electrode. Each configuration may be advantageous over the other depending on the position of the tumor on the test subject. Figure 10 below summarizes all of the general electrode configurations used by nanopulsers [3], [5], [10].

Figure 10: Summary of Electrode Configurations-Cuvette (top left), Microscope Slide (top right), 5-Needle Array (bottom left), Parallel Plate (bottom right)

Versatility of Designs

Nanopulser designs prove to be most advantageous when they permit a wide range of experimental versatility and robustness. The broader the versatility and adjustability of a generator, the greater the diversity of tests that can be conducted on cell suspensions. One of the disadvantages of typical nanopulse generator systems is that the electrical pulse duration is fixed by the length of the cable, mainly for Blumlein configurations [11]. The simplest way to amend this problem is to create a system where cables can be quickly interchanged. For example a generator called the "medipulser" described by [13] utilizes an interchangeable coaxial cable to adjust pulse lengths, and utilizes an adjustable oscillator to control pulse frequency.

One interesting pulse generator with an emphasis in versatility is one designed at the University of Limoges in France. This particular generator utilizes photoconductive semiconductor switches as switching elements, and uses coaxial cables to produce pulses, using the Blumlein principle. A physical representation of the system can be seen in figure 11 [8]. There are two basic components to the generator: a 1-port component on the left, and a 3-port component on the right. Between these two components, a coaxial cable of a chosen length for a desired pulse duration interconnects each component through the ports, and acts as the capacitive storage element. The right component's top port connects to a high voltage, DC power source, and the right component's right port connects to the load. Within each component is an embedded PCSS with an access aperture through which an optical laser beam can pass.

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Figure 11: A Versatile Nanopulser

The nanopulse generation process begins with both switches in the open position, allowing the central coaxial cable to be charged to the input voltage. Next, the right switch is triggered to the closed position, and two electromagnetic waves propagate in opposite directions. The pulse moving away from the load is completely reflected at the left boundary without any phase shift, and essentially increases the duration of the pulse that was initially moving towards the load. The amplitude of this pulse is half of the DC source's amplitude, and the total duration represents twice the amount of time required for a wave to travel down the transmission line. Bipolar pulses can also be produced if the left switch is closed in synchronization with the right switch [8]. Reference figure 12 by [8] to observe the two differing pulse shapes.

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Figure 12: Rectangular and Bipolar Pulses

This design is especially unique because it can easily produce pulses of multiple time durations by replacing the coaxial cable between the two components. The connection ports are configured in such a way to make transmission line interchanging quick and simple. Also, this configuration can produce two different types of pulse shapes, allowing additional experiments to be designed that explore the affects of pulse "shape" on cellular response. Lastly, the amplitude of the pulse can be adjusted by changing the input voltage to the generator. As a whole, this is one of the more robust generators that offer many versatile features that can serve a variety of applications [8].

Experimental Effects of Nanopulses on Cells and Tumors

Thanks to the creation of effective nanopulse generators, multiple experiments have been done concerning the effect of nanopulses on cells and tumors. One experiment, performed at Old Dominon University involved nanopulse application to a cell suspension in an electroporation cuvette. This particular study involved an analysis of membrane integrity after pulse application, comparing the effects of both nanopulses and microsecond-duration pulses. For each trial, the energy density of each pulse remained fixed, meaning that the electric field varied inversely with pulse length.

Propidium Iodide (PI) intake into the surface membrane was used to evaluate membrane integrity. Three observations were noted after the pulse train applications [3].

The first observation made by [3] was that delayed PI dye intake through the outer membrane occurred with shorter pulses. Distributions of the time needed for dye intake to occur can be viewed below in figure 13. The second difference observed by [3] was that long pulse trials tended to show a polarization at the anode side of the cell, while nanopulses caused dye intake to occur uniformly around the cell border. The third noted difference was that short pulses caused less cell swelling than long pulses [3].

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Figure 13: Dye Intake Distributions

The hypotheses reached by [3] as to why the differing effects occurred for different pulse durations conform with the earlier nanopulse theory previously described. It is inferred by the researchers that shorter pulses tend to affect the intracellular organelles and functions that ultimately cause cell apoptosis and permeabilization, rather than directly affecting the exterior membrane like longer duration pulses would tend to do [3].

In addition to nanopulse experiments on cell suspensions, studies have also been done within an in vivo test environment on live specimens. Another study conducted by the Old Dominion University Research Center for Bioelectrics used 120 live mice, each injected with melanoma tumors. 100 consecutive, 300 nanosecond-duration pulses with 40 kV/cm amplitude were applied to the each of the mice with either a 5-needle array electrode or a parallel plate electrode, with the tumor centered between the anode and cathode. Multiple test samples revealed the same data; within two days, the melanoma began shrinking and blood flow was disrupted to the tumor cells. With further application of nanopulses over a two week period, it was revealed that "multiple treatments resulted in complete tumor remission" [5]. The tumor remission process from the study by [5] can be seen in figure 14.

Figure 14: Treatment of Melanoma Tumors with Nanopulses

One of the significant observations that nanopulses seem to induce in cells and tumor tissue is the process of apoptosis. Apoptosis can be viewed or "programmed cell death" or "cell 'suicide' which occurs in response to stress through an orderly process of

morphological disintegration" [12]. Apoptosis, contrary to necrosis which is the process used by most cancer therapies, diminishes cancer cells "in the absence of any adverse inflammatory reaction, and allow[s], therefore, preservation of the integrity of the tissue, organ and whole organism in which cell death is occurring" [16]. It is apparent that nanosecond pulsed electric field treatments are presenting advantages and new areas of study concerning the treatment of cancerous tissue.

Conclusion

The design of nanopulse generators has been critical to bringing about a greater understanding of the subcellular effects caused by nsPEFs. While many forms of electricity-based treatments have been used in clinics, experimentation has suggested that electrical pulses in the nanosecond-duration have the potential to provide a treatment that does not rely on hyperthermia or toxic drugs to induce apoptosis. Further experimentation is likely to be done concerning nanopulses, and it is critical that nanopulsers be designed to facilitate productive experiments.

It is important when designing a nanopulse generator to fully understand the nature of the experiments in which it will be used, and to fully define the requirements and constraints, especially concerning the various pulse parameters like shape, amplitude, and duration. Various nanopulse generator topologies like the Blumlein or Marx bank scheme should be referenced in order to generate effective, ultrashort pulses, and careful detail must be put into the design and materials needed for the generator's electrical switch and electrode configuration. Precautions need to be taken to prevent unwanted electromagnetic effects such as reflection caused from impedance mismatch between the generator output and biological load. Lastly, a high degree of versatility for the

nanopulser is advantageous when a generator is required to meet multiple functions and allow parameter adjustment. While more research needs to be done to fully understand the mechanism of action behind nanopulse-induced effects, nanopulse cancer treatment certainly has potential to positively impact cancer patients around the world, as long as nanopulse generators continue to deliver the needed requirements.

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