Journal of
Tumor Medicine & Prevention
ISSN: 2575-890X

Juniper Publishers
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Editorial
Volume 4 Issue 1 - July 2023
DOI: 10.19080/JTMP.2023.04.555628

J Tumor Med Prev

Nanosecond Pulsed Electric Fields (nsPEFs) Enhance Pulsed Power Applications for Biology and Medicine

Stephen J Beebe*

Center for Bioelectrics, Old Dominion University, USA

Submission: July 17, 2023**; Published:** July 25, 2023

***Corresponding author:** Stephen J Beebe, Center for Bioelectrics, Old Dominion University, USA, Email: sbeebe@odu.edu

Editorial

The use of nanosecond pulsed electric fields (nsPEFs) for biological and medical applications is based on pulsed power technology. Pulsed power is the accumulation and storage of electrical energy in a bank of capacitors and the rapid discharge of it instantly, in short durations, thus increasing instantaneous high power without heating. It has been used for decades in pulsed power engineering, such as high-power lasers and particle accelerators, radar and communication systems, and high-voltage and power electronics. The use of pulsed power beyond pure physics presents new landscapes for this technology in biology and medicine.

During the inventions of science, advances in physics, especially in astronomy, preceded advances in biology and medicine by hundreds of years Wootton [1]. It seems during the discoveries in science, humankind found it easier and more interesting to look outward rather than inward. This is understandable given the millions of visible heavenly bodies requiring magnification to overcome distances. In biology, magnification was required to overcome small sizes of cells that were invisible to the naked eye Mukherjee [2]. Nevertheless, it is satisfying to see that in the case of electric fields, physics evolved into and within the fields of biology and medicine providing unique collaborations of disciplines.

The nsPEF predecessor, electroporation (EP) or electropermeabilization, used pulsed power released in microseconds or milliseconds to deliver DNA to cells Neumann et al [3] and enhanced the effect of poorly permeable therapeutic drugs in electrochemotherapy (ECT) Miklavcic et al [4] and in the delivery of DNA plasmids in gene electrotherapy (GET) Jaroszeski et a [5]. EP creates temporary pores in the plasma membrane of cells expediting the delivery of impermeable drugs and DNA into cells. Pulsed power is also used in plasma medicine with potential therapeutic effects of cold plasma or ionized gas for sterilization, wound healing, EP, and tumor treatment Zhai et al [6].

For nsPEFs and cold plasma, the pulse duration is decreased into the nanosecond range. To better understand pulsed power, consider a joule of energy stored and released in short time durations. If a joule of energy is stored and released in 1 second, it provides 1 watt of power. If released in a microsecond, it provides a megawatt of power. If released in a nanosecond, it provides a gigawatt of power. In most of our studies, nanosecond pulses include around 100 ns durations, which provides around 10 megawatts/pulse. After 1000 pulses at 50 kV/cm, a tumor can be safely eliminated. This non-thermal power is delivered through electrodes and the effects occur specifically within the domains of the electrodes, so it provides control of the intended effects without causing collateral damage. Yet amazingly, it takes a protocol of 1000 pulses at about 3 Hz at 100 ns duration and 50 kV/cm to eliminate tumors. That's a total of 10 gigawatts of power into a 5 cm tumor in a mouse breast or a rat liver delivering a charging effect of 5 volt-seconds/centimeter (Vs/cm).

So, for a period of about 5.5 mins, tumors see one thousand 100 ns pulses exposing tumors or cells to about 100 µsec of total electric field exposure time of 10 gigawatts delivered directly to the cells or tumor without collateral damage. Interestingly, in an orthotopic rat liver cancer model Lassiter et al [7] and an orthotopic mouse breast cancer model Guo et al [8], Beebe et al [9], the tumor-free rodents showed a vaccine effect, which is equivalent to in situ vaccination by the nsPEF treatment. That is when challenged 7 weeks after the nsPEF treatment with the tumor cell injection again, they rejected the tumor cells and did not grow a tumor. In both of these models, the immune

phenotypes that allowed these in situ vaccinations were defined. In general, the immunosuppressive tumor environment (TME) was eliminated and there were active CD8+ and CD4+ effector memory and central memory T-cells, and natural killer cells (NKs) in the liver model but not so much in the breast cancer model. However, not all nsPEF-treated cancer models exhibit this in situ vaccination response. For example, in ectopic mouse melanoma Rossi et al [10] and pancreatic cancer models Guo et al [8], only a few animals rejected the tumor challenge as a vaccine effect. One of the outstanding problems here is the continued presence of immunosuppression in the TME Guo et al [11].

It would appear that there are two thresholds or two types or targets that must be activated to induce tumor-free animals on one hand and tumor-free animals that are in situ vaccinated on the other. It is hypothesized that the vaccine effect is due to an "optimal" type of nsPEF-induced regulated cell death (RCD), said to be immunogenic, which allows dendritic cells (DCs) to properly sense cancer antigens to cross-present them to T-cells Galluzzi et al [12]. NsPEFs were shown to induce intrinsic apoptosis in Jurkat cells with caspase-3 and -9 activation and cytochrome c release into the cytoplasm Beebe et al [13]. Since we were looking for apoptosis in those studies, it is fortunate that we chose to use Jurkat cells because nsPEFs do not induce apoptosis in all cell types. Later we showed in Jurkat cells that nsPEFs induced caspase-dependent apoptosis at lower electric fields and caspase-independent RCD at higher electric fields Ren et al [14]. In HCC1937 human triple-negative breast cancer (TNBC) cells, nsPEF induced two different RCD programs of necroptosis and parthanatos Beebe [15]. Like the Jurkat cell studies, it was not possible to determine if both these RCD mechanisms were operative in the same cells or if the cell type could use two different mechanisms for RCD induction.

Parthanatos is a process dependent on the activity of poly (ADP-ribose)-polymerase (PARP), which is caused by DNA damage. It occurs by poly (ADP-ribose) (PAR) produced through PARP overactivation, causing a massive scale DNA fragmentation and chromatin condensation. It does not require caspase activation but includes PAR accumulation, which is a positive indicator of parthanatos, and depletion of nicotinamide adenine dinucleotide (NAD+), and adenosine triphosphate (ATP) leading to cell death Huang et al [16].

Interestingly, in vitro, nsPEFs do not induce apoptosis in N1-S1 liver cancer and 4T1-luc breast cancer, the two cell types that induce tumor elimination with vaccine effects and in situ vaccinations. Others have indicated that nsPEFs induce necrosis in B16f10 cells, as defined as a late cell death that did not involve caspase-3 activation Rossi et al [10]. However, necrosis does not tell us anything about the cell death process, only that the cells have died; necrosis means dead. If a cell does not die immediately by accidental cell death, they have been working to survive or to induce an alternative process of RCD. Both survival and cell death mechanisms are regulated.

There are at least two RCD pathways that result in the rupture of the cell plasma membrane, which can appear as accidental cell death (ACD) or necrosis. However, this rupture in RCD is due to the formation of intracellular structures that form pores in the plasma membrane. These are sometimes referred to as regulated necrosis. One of them is pyroptosis Bergsbaken et al [17], which is a highly inflammatory mode of RCD. Here caspase 1 is activated to cleave the protein gasdermin, which forms pores in the plasma membrane. Necroptosis Dhuriya and Sharma [18] is another inflammatory form of RCD that occurs under certain conditions, such as when apoptosis is inhibited or not properly executed. In necroptosis, receptor-interacting protein kinase 3 (RIP3) is activated by different signals and then recruits mixed lineage kinase domain-like pseudokinase (MLKL) and other components that form a complex or platform called the necrosome where MLKL is phosphorylated by RIP3. MLKL's conformation is changed, and it is released from the necrosome to form oligomers that ultimately translocate to the plasma membrane and lead to membrane disruption. Interestingly, necroptosis-deficient cells are poorly immunogenic and avoid natural and therapy-induced immunosurveillance Galluzzi et al [12]. This suggests that RCD by necroptosis may lead to vaccine effects and in situ vaccination. It will be interesting to determine if nsPEFs induce necroptosis in N1-S1 liver or 4T1-luc breast cancer. Although necroptosis was found in these human cells, it is not possible without humanized mice to determine if this mode of necroptosis induces a vaccine effect. While these antibodies for phosphor-RIP-3 and phosphor-MLKL are available for these human proteins, they have been difficult to find for the mice and rats phosphor-proteins.

In more recent investigations, observations indicate that with low charging nsPEF charging conditions it is possible to induce non-lethal cell processes. For example, we found that low-charging nsPEF treatment of mouse dendritic cells induces cell maturation as defined by the induced expression of activation markers on the DC membranes Guo et al [8]. This lead to the hypothesis that in addition to inducing an "appropriate" RCD mechanism in animals, it may also be possible for nsPEFs to activate DCs in the TME. This concept could come about by the presence of heterogeneous nsPEF treatment zones like that used in the rat liver studies employing a 5-needle electrode array with 4 ground needles forming a near square around a center positive electrode, which pierced the tumor center Chen et al [19]. In this design, regions between and adjacent to the high electric field zones, mostly around the electrode needles, and in zones in the periphery of the needles there are lower electric fields, where DCs could be induced to mature by lower charging conditions. Such a scenario would provide advantageous conditions for an optimal RCD mechanism in addition to the activation of DCs for tumor antigen recognition. This is only a hypothesis for the best of all worlds in a TME that could be tumor lethal and immunogenic. The activation of DCs is not the only example of noon-lethal activations. For example, others have also shown low charging nsPEFs induced proliferation and differentiation responses Vadlamani et al [20],

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Zhang et al [21], Zhang et al [22], Li et al [23]. Thus, in addition to exploring mechanisms of RCD, immunogenicity, immunity, and low charging effects of nsPEFs, this field of pulsed power is ripe for spreading physics deeper into biology and medicine.

Acknowledgment

The author extends thanks to Dr. Siqi Guo, Dr. Anthony Nanajian, Dr. Britt Ruedlinger, Dr. Shu Xiao, Dr. Wei Ren, Mr. Kamal Asadipour, Ms. Britany Lassiter, and Ms. Nova Sain as colleagues who have been part of the continued effort to define the mechanisms of nsPEF actions on cells and tumor and to characterize the mechanisms of immunity.

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