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Review Article

Considerations for Exploring Nanosecond Pulsed Electric Fields (nsPEFs) for Treatments of Cancer, Benign Skin Diseases, Atrial Fibrillation, and for New Mechanistic Understandings

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Abstract

Pulsed power includes acquiring electrical energy, compressing it, and releasing it in instantaneous bursts that are low in energy but very high in power. When the pulse duration is near the plasma membrane charging time constant, which is the time during which the cell interior is exposed to the applied pulsed electric field, it affects intracellular structures and functions. The technology is called nanosecond Pulsed Electric Fields (nsPEFs), nanosecond electric pulses (nsEP), or Nanopulse Stimulation (NPS™) according to Pulse Biosciences, Inc., a company taking the technology to the market. Initial studies showed the elimination of tumor cells *in vitro* by apoptosis, and other regulated cell death mechanisms, elimination of rodent and canine osteosarcoma, and a basal cell carcinoma clinical trial. In the rat liver and mouse breast cancers, tumor-free animals were *in situ* vaccinated (ISV), preventing the recurrence of the treated cancers. The technology has also focused on treating benign skin diseases, with some advantages over cryoablation. More recently, the same technology called nanosecond pulsed-field ablation (nsPFA) has been used to treat cardiac arrhythmias like Atrial Fabulation (AFib) with catheters in humans. In pre-clinical studies and now in humans, this technology is showing advantages over radiofrequency ablation and cryoablation. On a new mechanistic landscape, nonlethal nsPEFs modulation of electron transport in the plasma membrane and the mitochondria show potential for controlling redox homeostasis and metabolism. Furthermore, different nsPEF waveforms have different effects on cells; waveforms can differ by pulse duration, rise time, electric field, and/or post-pulse features. So, for nsPEFs, there is a lethal side used for ablation as with NPS and nsPFA and a more recently recognized nonlethal side indicating new possibilities to differentially modify cell physiology depending on the different nsPEF waveforms.

Introduction

Since the beginning of this century, studies using pulsed power with nanosecond Pulsed Electric Fields (nsPEFs) have expanded beyond general high-powered physics with pulsed-powered applications in biology and medicine. Short-duration, pulsed electric fields with short and/or fast rise times take the electric fields through cells, affecting intracellular structures and functions [1]. These shorter-duration pulses are unlike longer pulses typical of electroporation, which go around cells, primarily affecting the plasma membrane [2]. It is considered that the efficacy of nsPEFs for treating cancer is enhanced by intracellular effects that are enabled by these short durations and short or fast rise time pulses. As will be discussed below, these intracellular effects induce Regulated Cell Death (RCD) in tumor cells and host somatic cells in the Tumor Microenvironment (TME) that can lead to stimulation of host immune responses in some cancer models. This is facilitated by the intracellular effects of the nanosecond electric duration pulses [1]. They have also been shown to target cellular components in the epidermis and dermis that induce RCD without affecting surrounding fibrous and cellular

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components. More recently, they have been used to irreversibly permeabilize cell membranes killing myocytes without affecting the extracellular matrix and surgically ablating cardiac tissue that create durable transmural lesions that terminate recurrent arrhythmias. NsPEFs can also modulate non-lethal cell activities related to reduction-oxidation (redox) signaling events that determine cell function and fate [3]. Notably, this pulsed power technology with nsPEFs can ablate tumors and cardiac tissues. It can yet be used safely on the skin without scaring and induce non-lethal mechanisms in cells that can modify their functions. These uses demonstrate a novel, non-thermal technology without drugs that can have broad applications in medicine and biology far beyond those tasks in military and industrial applications for which it was initially designed.

NsPEFs induce immune responses and *in situ* vaccination (ISV) in two rodent cancer models

Initial studies found that nsPEFs could eliminate tumors [4-6], and many studies demonstrated several different tumor types could be eliminated by nsPEFs [7,8]. During these studies, nsPEFs were also found to protect animals from a post-ablation challenge injection of the same tumor types acting as a vaccine-like effect that can be described as *in situ* Vaccinations (ISV) against N1-S1 rat liver [9] and 4T1-luc mouse breast cancers [10,11]. There were also some successes in a Pan02 pancreatic model [12]. In the liver and breast cancer models that can be described as immunotherapy, immune mechanisms showed clear evidence of CD8+T-cell and/or Natural Killer (NK) cell activation and most importantly an elimination of the immunosuppressive cells in the TME and draining lymph nodes. In our experience, two other models, Pan02 pancreatic cancer [13] and B16f10 melanoma show tumor elimination but without ISV [14,15]. In the Pan02 model, there were insufficient vaccine-like protective effects (1/15) and abscopal effects (1/10). An analysis of the immune landscape showed that the numbers of both T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs) in blood were significantly reduced, but memory (CD44+) T-cells were absent. Furthermore, the numbers of Tregs and MDSCs did not reduce in spleens. Very few T-cells, but large numbers of MDSCs were present in the NPS treated TME. These results suggested that NPS-induced immune mechanisms in this model were limited [13]. In the B16f10 model, there was evidence of T-cell activation; however, immunosuppressive cells disappeared on post-treatment day 1 but returned on post-treatment days 3 and 7 [15]. So, the most likely explanation for the absence of an immune response and ISV in Pan02 and melanomas is the continued presence of immunosuppressive cells, regardless of the presence of CD8+ T-cells or NK cells. In this environment, even activated T-cells may be ineffective in attacking tumor cells. The immunosuppressive cells must be eliminated, which is the typical problem in most immunotherapy treatments.

Another study with the Pan02 pancreatic tumor model [12] showed that NPS alone eliminated as much as 80% of primary tumors and inhibited tumor growth in the rechallenge (40% - 50%). The addition of resiquimod, a toll-like receptor agonist, was effective in combination with NPS for eliminating the primary tumors and inhibiting tumor growth in the rechallenge (75%), demonstrating a vaccine-like effect. Depleting CD8+ cells reduced inhibition of the rechallenge injection by 35%. Furthermore, rechallenged tumors had 3-fold more CD8+ T-cells than tumors rechallenged after primary tumor resection. These findings suggested a long-term immune response had been stimulated. They also demonstrated that injection of OX40 at the time of NPS treatment was effective at eliminating the growth of untreated abscopal tumors.

NsPEF treatment (also called Nano-Pulse Stimulation™, NPS) for canine spontaneous osteosarcomas

In addition to treating smaller tumors in rodents, NPS with parallel dual needle electrodes successfully eliminated 75% (3/4) of canine spontaneous osteosarcomas with no cardiovascular events (supraventricular tachycardia, atrial fabulation pneumothorax or lower limb thrombosis) and no hyperthermic damage [16]. All control animals were euthanized with tumor sizes larger than 5 cm and/or lung metastasis causing respiratory difficulty. Elimination of osteosarcomas resulted in a reduction of metastasis with only one dog (1/4) showing lung metastatic disease. Although there was one transient capsular infection along a needle tract that recovered, there were no joint capsular deformities and all tumor-free dogs exhibited normal range of motion.

There is considerable heterogeneity of metabolic programs running in the tumor microenvironment, with competition between tumor cells and T-cells, both requiring glycolytic programs for cell expansion and T-cell function [17]. Serum alkaline phosphatase is an indicator of tumor metabolic activity and increases in this marker increased in osteosarcoma-bearing, untreated dogs. Treatment of canine osteosarcoma with NPS resulted in a decrease in alkaline phosphatase activity indicative of regressing tumor activity. So generally, nsPEFs extended survival and improved the quality of canine life by eliminating tumors and reducing metastasis in spontaneous canine osteosarcoma.

Clinical trials with nanosecond pulsed electric field therapy for Basal Cell Carcinoma (BCC)

The first-in-human clinical trial for nsPEFs, called nanoelectroablation, treated 10 BCC in three subjects [18] Seven of the 10 BCC were completely free of basaloid cells when biopsied, and two partially regressed. Two of the 7 exhibited seborrheic keratosis in the absence of basaloid cells. One of the 10 treated lesions recurred by week 10 and histologically had the appearance of squamous cell carcinoma. The study demonstrated that nanoelectroablation (nsPEFs, NPS) was safe and efficacious in treating human tumors. The advantages of this therapy over surgical excision or electro-desiccation and curettage are reduced pain, short treatment time, and the absence of scarring and multiple treatments. Furthermore, the procedure is non-thermal so burning is absent.

Later a multicenter prospective feasibility study of nsPEFs called nano-pulse stimulation™ technology was carried out for the treatment of nodular and superficial low-risk basal cell

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carcinoma [19]. The study was carried out using the CellFX® System using NPS™ technology from Pulse Biosciences. The endpoint of complete histological clearance of the target lesion was based on microscopic evaluation of Hematoxylin and Eosin (H&E) stains as evaluated by an independent board-certified dermatopathologist at 60 days post-CellFX treatment. Treatments of nodular and superficial low-risk Basal Cell Carcinomas (BCC) were effectively eliminated by NPS showing complete histological clearance in 92% (34/37) of cases. The other three cases were residual BCC, which most likely did not receive complete NPS coverage. The pain was effectively managed by intralesional injection of lidocaine during the CellFX treatment procedure, with the average subject reported pain rated as mild (2/10 standardized 11-point pain scale). Erythema, hyperpigmentation, and scaling were the most common skin effects observed 60 days post-CellFX treatment. Scaring was suspected due to the pretreatment biopsy rather than the CellFX treatment since the 5mm margins for the treatments were free of scar tissue. Investigators rated 95% of lesions as cosmetically acceptable. The majority (89%) of CellFX treated areas are expected to look better than curettage and electrodesiccation and 78% are expected to look better than standard surgical excision. In summary, the CellFX System is safe and effective for the treatment of low-risk nodular and superficial BCC and may be an emerging, non-surgical treatment option for the treatment of primary BCCs requiring maximal sparing of tissues, including facial lesions.

NsPEFs from benign skin diseases

NsPEFs have also been used to successfully treat benign skin diseases in clinical trials for epidermal lesions such as Seborrheic Keratosis Hyperplasia (SGH) [20], seborrheic keratoses [21], and non-genital, cutaneous verrucae (warts) [22]. For these lesions, the NPS is delivered through a parallel linear array of six microneedles 5 mm apart spanning a width of 5 mm. The tips are 2 mm or 3 mm long and penetrate the reticular dermis. The energy delivered is a product of the pulse duration, the electric field, and the current with much less energy delivered than by micro- or milli-second pulses providing non-thermal treatment. The short pulse duration and fast rise time allow the electric fields to enter cells affecting intracellular structures and functions while creating nanopores in the plasma membrane and intracellular membranes. In the SGH study, all lesions were located on the face, with 53% of them on the forehead and 46% on the cheeks or chin. Subjects rated their level of discomfort on a 10-point pain scale after the treatment of each lesion. Lidocaine was used as an anesthesia. Most subjects reported no pain (29%) or mild pain (54%). Fifteen percent reported moderate-severe, 1% reported very severe, and no subjects reported Worst Possible Pain. Sixty days after treatment 90.3% were cleared and 9.5% mostly cleared. For lesions receiving both 1 and 2 nsPEF treatments, hyperpigmentation peaked at the 30-day visit, and decreased with time. By the 60-days, 45% of lesions exhibited some degree of hyperpigmentation. Erythema was usually present 5 days after treatment, decreased substantially by 30 days, and was only observed in 7% of the treated lesions at 60 days. 5 days post-treatment only 37% of lesions showed swelling and this decreased to 1% at 30 days. No swelling was observed at 90 days. Overall, 101 of 222 treated lesions (45%) showed some degree of hyperpigmentation at the last observation available. These trials show that the nsPEF procedure provides a rapid safe and effective treatment for SGHs with a low risk of scarring and long-term hyperpigmentation. The SGH clearance is highly localized with no systemic side effects. The mechanism of nsPEF therapy targets cellular structures within the epidermis and dermis. Therefore, it is ideal to eliminate sebaceous glands with a high clearance rate, and high degree of subject satisfaction.

Safety and efficacy were also shown for the treatment of Seborrheic Keratosis (SK). Again, the short duration and fast pulse rise time enable the electric fields to penetrate the cell interior affecting the mitochondria and endoplasmic reticulum, as well as the plasma membrane. NPS is safe and effective specifically affecting cellular structures including the epidermis with minimal effects on acellular structures in the dermis. NPS is superior to common methods used on SK including curettage, electrodesiccation cryosurgery, and chemical (high concentration of hydrogen peroxide) destructions of laser ablation avoiding recurrence, scaring, and pigmentation changes.

NPS has also been shown to be safe and effective for successfully treating non-genital warts (verrucae), caused by HPV-infected epidermal cells [22]. Applicator tips (five different designs) included two parallel rows of 2-mm-long microneedles for the three smaller sizes and three rows for the two larger sizes through which 200 ns pulse durations and bipolar pulses were generated by the CellFX system. All warts were treated with lidocaine before treatment. Treatment site reactions were like those of SGH and SK lesions. Warts were treated on the back, feet and most (50%) on the hands. Seventy-five percent of warts were cleared with one or two treatments, which is fewer than generally required with cryotherapy. Further, 65% of recalcitrant warts were effectively removed. None of the 195 warts recurred after 120 days of observation, indicating lower recurrence with NPS than other treatment therapies, which resolves a major problem with wart treatment. Interestingly, 24.5% of untreated control warts were cleared, suggesting a bystander effect from a possible immune response. This is reminiscent of NPS treated HPV -transformed tumors in mice where 25% were resistant to challenge injections of the same tumors suggesting a vaccine-like effect in a CD8+ T-cell-dependent manner suggesting an immune response in some mice after NPS [23].

NsPEF cardiac ablation for atrial fibrillation

NsPEFs have also been investigated for non-thermal cardiac ablation [24-26] and treatment for atrial fibrillation, flutter, and ventricular tachycardia as an alternative to radiofrequency ablation (RFA), which has significant acute success rates [27]; However, RFA suffers from high recurrence rates within months or years, which is attributed to gaps in the lesions [28,29]. RFA occurs by resistive heating and blood vessels can serve to cool the ablation mechanism, which limits the consistency of RFA lesions [30]. The heat is also responsible for complications

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(4%) that are not trivial including stroke, tamponade, vascular injury, pulmonary vein occlusion, and atrial esophageal fistulae [31-33]. NsPEFs penetrate deeper and the electric fields are less affected by tissue electrical inhomogeneities [34-36].

Studies have been conducted in rabbit hearts [24] with needle electrodes and in swine hearts with clamp electrodes [25,26] like those used in RFA and cryoablation. The studies in rabbit hearts created smoother more uniform lesions than RFA that were transmurally deep, and the width was controlled by the spacing of the electrodes with minimal heating (<2 C). Studies in the swine confirmed the finding from the rabbit studies showing that all lesions were transmural deep and with highly consistent widths across the wall. There were no pulse-induced arrhythmias or other complications. The pig studies also demonstrated that the lesions were non-conducting for 6 weeks and 6 months post-ablation. The lesion volumes were filled mostly with collagen and variable amounts of fat tissue, high in some lesions. The lesions were non-proliferative shown by Ki-67. There were some smooth muscles (smooth muscle actin-positive) that either survived or recovered. Most of the lesions had no, minimal, or thin residual muscle that was connexin-43-(cardiac gap-junction protein)-negative indicating the absence of conductivity. Overall the studies show that nsPEFs can be a safer more effective treatment for atrial fibrillation that requires cardiac ablation, with faster treatment times with lesions showing non-conductivity for at least 6 months. Clinical trials are in progress but no published studies yet. Human clinical trials with cardiac ablations are in progress, but none have been published yet.

(New) mechanistic understandings of nsPEF Effects

While nsPEFs have several possible clinical applications, a major topic of interest has been the mechanistic impact of nsPEFs upon cells as the means for cellular and tumor consequences. An early study demonstrated that nsPEFs induced apoptosis in Jurkat cells as demonstrated by cytochrome c release from the mitochondria into the cytoplasm and by caspase-3 activation [37]. It was later shown using an APAF-1 knockdown (APAF-kd) Jurkat clone, that nsPEFs induced caspase-dependent apoptosis at lower electric fields and caspase-independent apoptosis at higher electric fields, showing that nsPEF-induced cell death was electric field-dependent and that more than one Regulated Cell Death (RCD) mechanism could occur in each cell type [38]. NsPEFs also induced dissipation of the mitochondrial membrane potential ($\Delta\Psi m$) regardless of the presence of caspase activation as shown by the absence of differences $\Delta\Psi m$ losses in the wild-type and APAF-kd clones [39]. Two modes of cell death were also shown in U937 cells described as necrosis (perhaps accidental cell death (ACD) or undefined RCD) followed by apoptotic cell death [40]. It was shown that nsPEFs induced necroptosis and parthanatos in human HCC1937 triple-negative breast cancer cells [39]. Generally, it can be considered that nsPEF-induced cell death is cell type-dependent. Interestingly, in the liver [9] and breast [10] cancer models where nsPEFs induce ISV, neither of them appears to undergo cell death by apoptosis. This may be really expected since apoptotic cell death is silent so a minimum of cancer antigens would be released and identified during apoptotic cell death.

However, there is much more to nsPEFs than meets with strict ablation applications. It is known that the dissipation of the $\Delta\Psi m$ is enhanced by $Ca2+$ [38,41]. It was also recently shown that nsPEFs induce the formation of reactive oxygen species (ROS) differently in cancer and non-cancer cells. In 4T1-luc mammary cancer cells, there was greater ROS production in mitochondria detected by MitoSox In contrast, non-cancer H9c2 cardiac myoblasts produced more ROS in the cytoplasm detected by DCFDA [42]. This is metabolically reasonable since cancer cells depend more on glycolysis for their growth and expansion while cardiac cells depend more on oxidative phosphorylation for ATP production and cardiac function.

Another newly recognized property of nsPEFs is their capacity to modulate Electron Transport (ET) in the mitochondria and in the Plasma Membrane Redox System (PMRS) [42]. In the mitochondria, nsPEFs attenuate oxygen consumption as a determinant of ET. This diminution of ET occurs in the basal state and when the mitochondria are uncoupled from oxidative phosphorylation. This attenuation of ET (oxygen consumption) occurs at least at complex I, which is known to be a major mitochondrial site for ROS production [43] and a likely source of mitochondrial ROS in response to nsPEFs.

NsPEFs also regulate trans-Plasma Membrane Electron Transport (tPMET) rates in the PMRS shown as a reduction of the cell-impermeable, WST-8 tetrazolium dye. The tPMET rates are increased at lower electric fields and decreased at higher electric fields when pulses are produced by a Pulse-forming Line (PFL) generator. Therefore, when non-lethal electric field conditions are used, nsPEFs can modulate redox homeostasis and metabolic reactions. Given that immune cell function is dependent on metabolic state, this provides a means for affecting immune cell function. It has been demonstrated that nsPEFs induced maturation (activation) of bone marrow-derived dendritic cells as indicated by the expression of co-stimulatory molecules on their cell surfaces [10]. This has also been demonstrated in human monocyte-derived DCs [Lassiter and Beebe, unpublished].

Since our initial and early studies generally focused on tumor elimination and mechanisms of cell death, we focused on effects with higher electric fields. However, as mentioned above at lower electric fields, nsPEFs show that non-lethal effects of nsPEFs require further study. This “softer side” of nsPEFs can be further exploited considering that the quantity and quality of the nsPEF stimulus are dependent on the nsPEF waveform [38,44.45]. NsPEF effects have almost exclusively been shown to be associated with nsPEF waveforms defined by pulse duration, rise time, amplitude (electric field), and pulse number. For example, by decreasing the pulse duration to near the plasma membrane charging time constant [1], nsPEFs induce greater effects on intracellular calcium mobilization [44] and by increasing the pulse rise-fall time, nsPEFs induced increased effects on the $\Delta\Psi m$ and cell death [38]. Other factors, such as low-intensity post-pulse waveform, had not been

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considered. However, the post-pulse waveforms can alter the cell responses produced by the primary pulse waveform and can even elicit unique cellular responses. For example, using two common pulse generator designs, including the Blumlein Line (BL) and the Pulse Forming Line (PFL) generators, both featuring nearly identical 100 ns pulse durations, evoke unique post-pulse cellular effects despite the primary pulse waveform being nearly identical [45]. For example, the effective nsPEF condition for a 50% effect (IC_{50}) for dissipation of the $\Delta\Psi m$ with the PFL and BL generators were 0.013 and 0.017 Vs/cm (see footnote), respectively, Surprisingly the IC_{50} for increase in ROS was 0.011 Vs/cm for both pulse generators. Thus, both pulse generators produced the same levels of ROS with different effects on the $\Delta\Psi m$. This raises questions about the dependence for loss of $\Delta\Psi m$ caused by ROS in response to nsPEFs.

Considering the effect of these pulsers on the tPMET, only the PFL generator exhibited a biphasic effect with an increase at lower electric fields and a decrease at higher electric fields. The BL generator only showed a decrease in the tPMET at higher electric fields. When effects on metabolism in the Seahorse stress test were analyzed 15 hr. after nsPEF treatment, there were no effects in basal metabolism with either pulser. However, the BL had no significant effect on spare respiratory capacity (SRC), while the PFL significantly decreased the SRC [45]. Thus, it is anticipated with further study, there will be other differences in the metabolic effects among cell types between the two pulsers among other different nsPEF waveforms. The PFL had a more detrimental effect on cell viability than the BL generator with IC_{50} for cell death were 0.012 *vs.* 0.015 Vs/cm, respectively. Our *in vivo* studies on tumor elimination [8] and ISV [9-11] are carried out with BL generators. These *in vitro* IC_{50} studies suggest that tumor treatment with a PFL generator might be more effective for tumor elimination than BL generators. However, the PFL requires higher electric fields, which might not be optimal in clinical settings.

Overall, the non-lethal studies reviewed here indicate that there are new horizons for exploring nsPEF waveforms for differential effects in cell functions. Given the findings that nsPEFs can not only eliminate tumors but can also lead to immune responses that lead to ISV, at least in some tumor models, consideration of specific immune cell metabolic programs, which are required for specific immune cell functions, may enlighten our understanding of how nsPEFs can induce immune responses. However, because not all cancer models exhibit nsPEF immune responses and ISV this requires further exploration. The finding that a PFL generator induced mouse bone marrow-derived dendritic cell maturation suggests that specific metabolic pathways had to be activated to provide the means for new co-stimulatory molecule production. Other studies have shown that non-lethal nsPEF induces proliferation and differentiation in osteoblasts and myoblasts [46]. These cell functions require different metabolic programs. In addition, nsPEFs enhanced mesenchymal stem cell differentiation via DNMT1-regulated OCT4/NANOG gene expression [47]. These findings indicate a more thorough analysis of nsPEFs on proliferation, differentiation, metabolism, and gene expression is appropriate. Importantly, studies for the effects of different nsPEF waveforms on multiple cell types require further exploration.

Conclusion

The uses of pulsed power with nanosecond pulse durations provide electric fields to go through cells affecting the plasma membrane and intracellular mechanisms as opposed to going around cells primarily affecting like pulses with microsecond or millisecond pulse typical of electroporation [36]. When the pulse amplitude is sufficiently high, cells die by regulated cell death mechanisms that are cell type dependent. This strategy has been used to eliminate cancer essentially of all types and in a few models, nsPEFs induce vaccine effects as ISV. This technology has also been extended for the removal of non-cancerous or benign lessons including epidermal lesions such as seborrheic keratosis hyperplasia, seborrheic keratoses, and non-genital, cutaneous verrucae (warts). Since the electric field areas are controlled by the placement of the electrodes it is possible to identify selective targets such as cellular components in the epidermis and dermis that induce RCD without affecting surrounding fibrous and cellular components. So, for these benign skin lesions, it is possible to specifically affect the epidermis with minimal effects on acellular structures in the dermis and without scarring. More recently, nsPEFs or nsPFA have been used to irreversibly permeabilize cell membranes killing myocytes without affecting the extracellular matrix and surgically ablating cardiac tissue that create durable transmural lesions that terminate recurrent arrhythmias such as atrial fibrillation, flutter, and ventricular tachycardia as an alternative to radiofrequency ablation (RFA), which has heating as an unwanted side effect. In addition to their ablation potential, nsPEFs can have significant effects that are non-lethal and affect cell fate. The finding that nsPEFs can modulate electron transport has a significant potential to modulate metabolism as shown by the activation of bone marrow-derived DCs, induce proliferation and differentiation in osteoblasts and myoblasts, and induce mesenchymal stem cell differentiation. These initial non-lethal findings and the concept that different nsPEF waveforms can modulate different cellular effects open new fields for exploration and possible clinical application to be resolved with further research.

Footnote

The charging effects of nsPEFs as Vs/cm are determined for *in vitro* experiments where cells are treated in suspension is determined as the pulse duration (τ) in seconds (s), times the electric field in volts/centimeter (v/cm) (E), times the square root of pulse number $(n)^{1/2}$ or Vs/cm = τ x E x $n^{1/2}$. The square root factor, only determined for *in vitro* experiments, includes the random walk theory [48]. The formula for *in vivo* tumor treatment is τ x E x n.

References

1. Schoenbach KH, Beebe SJ, Buescher ES. Intracellular effect of ultrashort electrical pulses. Bioelectromagnetics. 2001;22(6):440-448. Available from: https://doi.org/10.1002/bem.71

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2. Gowrishankar TR, Weaver JC. Electrical behavior and pore accumulation in a multicellular model for conventional and supra-electroporation. Biochem Biophys Res Commun. 2006;349:643-653. Available from: https://doi.org/10.1016/j.bbrc.2006.08.097

3. Beebe SJ, Schoenbach KH. Nanosecond pulsed electric fields: A new stimulus to activate intracellular signaling. J Biomed Biotechnol. 2005;2005:297-300. Available from: https://doi.org/10.1155/jbb.2005.297

4. Beebe SJ, Fox PM, Rec LH, Buescher ES, Somers K, Schoenbach KH. Nanosecond pulsed electric field (nsPEF) effects on cells and tissues: apoptosis induction and tumor growth inhibition. IEEE Trans Plasma Sci. 2002;30:286-292. Available from: https://doi.org/10.1109/TPS.2002.1003872

5. Nuccitelli R, Pliquett U, Chen X, Ford W, Swanson RJ, Beebe SJ, Kolb JF, Schoenbach KH. Nanosecond pulsed electric fields cause melanomas to self-destruct. Biochem Biophys Res Commun. 2006;343:351-360. Available from: https://doi.org/10.1016/j.bbrc.2006.02.181

6. Nuccitelli R, Chen X, Pakhomov AG, Baldwin WH, Sheikh S, Pomicter JL, et al. A new pulsed electric field therapy for melanoma disrupts the tumor's blood supply and causes complete remission without recurrence. Int J Cancer. 2009;125:438-445. Available from: https://doi.org/10.1002/ijc.24345

7. Beebe SJ. Preclinical studies with nanosecond pulses. In: Miklavčič D, editor. Handbook of Electroporation. Springer; 2016;1543-1562.

8. Beebe SJ. Ultrashort electric pulses in pre-clinical cancer treatment. In: Beebe SJ, Joshi R, Schoenbach KH, Xiao S, editors. Ultrashort Electric Pulses Effects in Biology and Medicine. Springer Nature Singapore; 2021. [Chapter 18]. Available from: https://www.springerprofessional.de/en/ultrashort-electric-pulse-effects-in-biology-and-medicine/19186724

9. Lassiter BP, Guo S, Beebe SJ. Nano-pulse stimulation ablates orthotopic rat hepatocellular carcinoma and induces innate and adaptive memory immune mechanisms that prevent recurrence. Cancers (Basel). 2018;10:69. Available from: https://doi.org/10.3390/cancers10030069

10. Guo S, Jing Y, Burcus NI, Lassiter BP, Tanaz R, Heller R, et al. Nano-pulse stimulation induces potent immune responses, eradicating local breast cancer while reducing distant metastases. Int J Cancer. 2018;142:629-640. Available from: https://doi.org/10.1002/ijc.31071

11. Beebe SJ, Lassiter BP, Guo S. Nanopulse stimulation (NPS) induces tumor ablation and immunity in orthotopic 4T1 mouse breast cancer: a review. Cancers (Basel). 2018;10:97. Available from: https://doi.org/10.3390/cancers10040097

12. McDaniel A, Rothstein KV, Gonzalez D, Nuccitelli R. Nano-pulse stimulation treatment inhibits Pan02 murine pancreatic tumor growth and induces a long-term adaptive immune response with abscopal effects when combined with immune-enhancing agents. Bioelectricity. 2024;6(2):108-117 Available from: https://doi.org/10.1089/bioe.2024.0011.

13. Guo S, Burcus NI, Hornef J, Jing Y, Jiang C, Heller R, Beebe SJ. Nano-pulse stimulation for the treatment of pancreatic cancer and the changes in immune profile. Cancers (Basel). 2018;10(7):217. Available from: https://doi.org/10.3390/cancers10070217

14. Rossi A, Pakhomova ON, Pakhomov AG, Weygandt S, Bulysheva AA, Murray LE, et al. Mechanisms and immunogenicity of nsPEF-induced cell death in B16F10 melanoma tumors. Sci Rep. 2019;9:431. Available from: https://doi.org/10.1038/s41598-018-36527-5

15. Ruedlinger BL. The investigations of NPS modulated immunity and immunometabolism [dissertation]. Norfolk (VA): Old Dominion University; 2021. Available from: https://digitalcommons.odu.edu/gradschool_biomedicalsciences_etds/12/

16. Chen X, Chen Y, Jiang J, Wu L, Yin S, Miao X, et al. Nano-pulse stimulation (NPS) ablates tumors and inhibits lung metastasis on both canine spontaneous osteosarcoma and murine transplanted hepatocellular carcinoma with high metastatic potential. Oncotarget. 2017;8:44032-44039. Available from: https://doi.org/10.18632/oncotarget.17178

17. Scharping NE, Delgoffe GM. Tumor microenvironment metabolism: a new checkpoint for anti-tumor immunity. Vaccines (Basel). 2016;4:46. Available from: https://doi.org/10.3390/vaccines4040046

18. Nuccitelli R, Wood R, Kreis M, Athos B, Huynh J, Lui K, et al. First-in-human trial of nanoelectroablation therapy for basal cell carcinoma: proof of method. Exp Dermatol. 2014;23:135-137. Available from: https://doi.org/10.1111/exd.12303

19. Ross AS, Schlesinger T, Harmon CB, Moy RL, Rohrer TE, Mehregan DR, et al. Multicenter, prospective feasibility study of Nano-Pulse Stimulation™ technology for the treatment of both nodular and superficial low-risk basal cell carcinoma. Front Oncol. 2022;12:1044694. Available from: https://doi.org/10.3389/fonc.2022.1044694

20. Munavalli GS, Zelickson BD, Selim MM, Kilmer SL, Rohrer TE, Newman J, et al. Safety and Efficacy of Nanosecond Pulsed Electric Field Treatment of Sebaceous Gland Hyperplasia. Dermatol Surg. 2020;46(6):803-809. Available from: https://doi.org/10.1097/dss.0000000000002154

21. Hruza GJ, Zelickson BD, Selim MM, Rohrer TE, Newman J, Park H, et al. Safety and efficacy of nanosecond pulsed electric field treatment of seborrheic keratoses. Dermatol Surg. 2020;46(9):1183-1189. Available from: https://doi.org/10.1097/dss.0000000000002278

22. Nuccitelli R, LaTowsky BM, Lain E, Munavalli G, Loss L, Ross EV, et al. Safety and efficacy of nano-pulse stimulation treatment of non-genital, cutaneous warts (verrucae). Lasers Surg Med. 2021 Dec;53(10):1301-1306. Available from: https://doi.org/10.1002/lsm.23423

23. Skeate JG, Da Silva DM, Chavez-Juan E, Anand S, Nuccitelli R, Kast WM. Nano-pulse stimulation induces immunogenic cell death in human papillomavirus-transformed tumors and initiates an adaptive immune response. PLoS One. 2018;13(1):e0191311. Available from: https://doi.org/10.1371/journal.pone.0191311

24. Xie F, Varghese F, Pakhomov AG, Semenov I, Xiao S, Philpott J, et al. Ablation of myocardial tissue with nanosecond pulsed electric fields. PLoS One. 2015;10:e0144833. Available from: https://doi.org/10.1371/journal.pone.0144833

25. Varghese F, Philpott JM, Neuber JU, Hargrave B, Zemlin CW. Surgical ablation of cardiac tissue with nanosecond pulsed electric fields in swine. Cardiovasc Eng Technol. 2023;14:52-59. Available from: https://doi.org/10.1007/s13239-022-00634-2

26. Serra F, Philpott JM, Neuber JU, Shih E, Etheridge JC, Varghese F, et al. Nanosecond pulsed electric field ablation with a bipolar clamp creates durable transmural lesions in cardiac tissue. Circ Arrhythm Electrophysiol. 2023;16: e012300. Available from: https://doi.org/10.1161/circep.123.012300

27. Gal P, Aarntzen AE, Smit JJ, Adiyaman A, Misier AR, Delnoy PP, et al. Conventional radiofrequency catheter ablation compared to multi-electrode ablation for atrial fibrillation. Int J Cardiol. 2014;176:891-895. Available from: https://doi.org/10.1016/j.ijcard.2014.08.034

28. Gerstenfeld EP, Callans DJ, Dixit S, Zado E, Marchlinski FE. Incidence and location of focal atrial fibrillation triggers in patients undergoing repeat pulmonary vein isolation: implications for ablation strategies. J Cardiovasc Electrophysiol. 2003;14:685-690. Available from: https://doi.org/10.1046/j.1540-8167.2003.03013.x

29. Yokoyama K, Nakagawa H, Wittkampf FH, Pitha JV, Lazzara R, Jackman WM. Comparison of electrode cooling between internal and open irrigation in radiofrequency ablation lesion depth and incidence of thrombus and steam pop. Circulation. 2006;113:11-19. Available from: https://doi.org/10.1161/circulationaha.105.540062

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30. Nakagawa H, Yamanashi WS, Pitha JV, Arruda M, Wang X, Ohtomo K, et al. Comparison of in vivo tissue temperature profile and lesion geometry for radiofrequency ablation with a saline-irrigated electrode versus temperature control in a canine thigh muscle preparation. Circulation. 1995;91(8):2264-2273. Available from: https://doi.org/10.1161/01.cir.91.8.2264

31. Bertaglia E, Zoppo F, Tondo C, Colella A, Mantovan R, Senatore G, et al. Early complications of pulmonary vein catheter ablation for atrial fibrillation: a multicenter prospective registry on procedural safety. Heart Rhythm. 2007;4:1265-1271.
Available from: https://doi.org/10.1016/j.hrthm.2007.06.016

32. Spragg DD, Dalal D, Cheema A, Scherr D, Chilukuri K, Cheng A, et al. Complications of catheter ablation for atrial fibrillation: incidence and predictors. J Cardiovasc Electrophysiol. 2008;19:627-631.
Available from: https://doi.org/10.1111/j.1540-8167.2008.01181.x

33. Woods CE, Olgin J. Atrial fibrillation therapy now and in the future: drugs, biologicals, and ablation. Circ Res. 2014;114:1532-1546.
Available from: https://doi.org/10.1161/circresaha.114.302362

34. Kotnik T, Miklavčič D. Second-order model of membrane electric field induced by alternating external electric fields. IEEE Trans Biomed Eng. 2000;47:1074-1081. Available from: https://doi.org/10.1109/10.855935

35. Gowrishankar TR, Weaver JC. An approach to electrical modeling of single and multiple cells. Proc Natl Acad Sci U S A. 2003;100:3203-3208.
Available from: https://doi.org/10.1073/pnas.0636434100

36. Gowrishankar TR, Esser AT, Vasilkoski Z, Smith KC, Weaver JC. Microdosimetry for conventional and supra-electroporation in cells with organelles. Biochem Biophys Res Commun. 2006;341:1266-1276.
Available from: https://doi.org/10.1016/j.bbrc.2006.01.094

37. Beebe SJ, Fox PM, Rec LJ, Willis EL, Schoenbach KH. Nanosecond, high-intensity pulsed electric fields induce apoptosis in human cells. FASEB J. 2003;17:1493-1495. Available from: https://doi.org/10.1096/fj.02-0859fje

38. Beebe SJ, Chen YJ, Sain NM, Schoenbach KH, Xiao S. Transient features in nanosecond pulsed electric fields differentially modulate mitochondria and viability. PLoS One. 2012;7:e51349.
Available from: https://doi.org/10.1371/journal.pone.0051349

39. Beebe SJ. Ultrashort electric pulses induce regulated cell death mechanisms. In: Beebe SJ, Joshi R, Schoenbach KH, Xiao S, editors. Ultrashort Electric Pulses Effects in Biology and Medicine. Springer Nature Singapore; 2021; [Chapter 9]. Available from: https://link.springer.com/book/10.1007/978-981-10-5113-5

40. Pakhomova ON, Gregory BW, Semenov I, Pakhomov AG. Two modes of cell death caused by exposure to nanosecond pulsed electric field. PLoS One. 2013;8:e70728.
Available from: https://doi.org/10.1371%2Fjournal.pone.0070278

41. Beebe SJ, Sain NM, Ren W. Induction of cell death mechanisms and apoptosis by nanosecond pulsed electric fields (nsPEFs). Cells. 2013;2:136-162. Available from: https://doi.org/10.3390/cells2010136

42. Asadipour K, Hani MB, Potter L, Ruedlinger BL, Lai N, Beebe SJ. Nanosecond pulsed electric fields (nsPEFs) modulate electron transport in the plasma membrane and the mitochondria. Bioelectrochemistry. 2024;155:108568.
Available from: https://doi.org/10.1016/j.bioelechem.2023.108568

43. Turrens JF, Boveris A. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochem J. 1980;191:421-427.
Available from: https://doi.org/10.1042/bj1910421

44. Semenov I, Xiao S, Pakhomova ON, Pakhomov AG. Recruitment of intracellular Ca2+ by ultrashort electric stimuli: the impact of pulse duration. Cell Calcium. 2013;54:145-150.
Available from: https://doi.org/10.1016/j.ceca.2013.05.008

45. Asadipour K, Zhou C, Yi V, Beebe SJ, Xiao S. Ultra-low intensity post-pulse affects cellular responses caused by nanosecond pulsed electric fields. Bioengineering (Basel). 2023;10:1069.
Available from: https://doi.org/10.3390/bioengineering10091069

46. Vadlamani RA, Nie Y, Detwiler DA, Dhanabal A, Kraft AM, Kuang S, Gavin TP, Garner AL. Nanosecond pulsed electric field induced proliferation and differentiation of osteoblasts and myoblasts. J R Soc Interface. 2019;16:20190079. Available from: https://doi.org/10.1098/rsif.2019.0079

47. Li K, Ning T, Wang H, Jiang Y, Zhang J, Ge Z. Nanosecond pulsed electric fields enhance mesenchymal stem cells differentiation via DNMT1-regulated OCT4/NANOG gene expression. Stem Cell Res Ther. 2020;11:308.
Available from: https://doi.org/10.1186/s13287-020-01821-5

48. Schoenbach KH, Joshi RP, Beebe SJ, Baum CE. A scaling law for membrane permeabilization with nanopulses. IEEE Trans Dielectrics Electr Insulation. 2009;16:1224-1235.
Available from: https://doi.org/10.1109/TDEI.2009.5293932

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