ANNALS OF MEDICINE
2022, VOL. 54, NO. 1, 1749–1756
https://doi.org/10.1080/07853890.2022.2087898

Taylor & Francis
Taylor & Francis Group

ARTICLE COMMENTARY

Nanosecond pulsed electric field (nsPEF) and vaccines: a novel technique for the inactivation of SARS-CoV-2 and other viruses?

A. R. Ruiz-Fernández[a,b], M. Rosemblatt[a,c] and T. Perez-Acle[a,b]

[a]Computational Biology Lab, Centro Ciencia & Vida, Fundación Ciencia & Vida, Santiago, Chile; [b]Facultad de Ingeniería y Tecnología, Universidad San Sebastián, Santiago, Chile; [c]Facultad de Medicina y Ciencia, Universidad San Sebastián, Santiago, Chile

ABSTRACT

Since the beginning of 2020, worldwide attention has been being focussed on SARS-CoV-2, the second strain of the severe acute respiratory syndrome virus. Although advances in vaccine technology have been made, particularly considering the advent of mRNA vaccines, up to date, no single antigen design can ensure optimal immune response. Therefore, new technologies must be tested as to their ability to further improve vaccines. Nanosecond Pulsed Electric Field (nsPEF) is one such method showing great promise in different biomedical and industrial fields, including the fight against COVID-19. Of note, available research shows that nsPEF directly damages the cell's DNA, so it is critical to determine if this technology could be able to fragment either viral DNA or RNA so as to be used as a novel technology to produce inactivated pathogenic agents that may, in turn, be used for the production of vaccines. Considering the available evidence, we propose that nsPEF may be used to produce inactivated SARS-CoV-2 viruses that may in turn be used to produce novel vaccines, as another tool to address 20 the current COVID-19 pandemic.

KEY MESSAGES

- Viral inactivation by using pulsed electric fields in the nanosecond frequency.
- DNA fragmentation by a Nanosecond Pulsed Electric Field (nsPEF).
- Opportunity to apply new technologies in vaccine development.

ARTICLE HISTORY
Received 5 March 2022
Revised 31 May 2022
Accepted 5 June 2022

KEYWORDS
nsPEF; vaccines; NPS; SARS-CoV-2

Introduction

Nanosecond Pulsed Electric Field (nsPEF) is a novel technology first developed in 1995 [1], exhibiting an explosive research growth since ~2005 [2]. This technique consists of the delivery of a series of pulses composed of high amplitude electric fields (~1–300 kV/cm) in the nano and sub-nanoseconds timescale into biological tissues or cells. Its primary effect on cells in the formation of membrane nanopores and the activation of ionic channels [3–14]. The main cellular consequence of nsPEF is the increment in the cytoplasmic concentration of Ca^{2+} [15–18], triggering signalling cascades ending either on apoptosis [19–25], or cell proliferation [26–28] and differentiation [29]. In contrast to other forms of electrostimulation, such as electroporation, nsPEF uses timescales similar to that of the charging time of cellular membranes (~100 ns in mammalian cells). This turns nsPEF capable of affecting inner organelles [15,30–33], including the nucleolus [30], making nsPEF a unique tool to manipulate cells. As different cell types have different membrane charging times and different conductivity in their surrounding media, the effects of nsPEF could be cell-type tailored. This characteristic has been exploited by researchers to propose a broad spectrum of nsPEF applications such as: neuron [7,11,34–37] and myocyte activation [38–41], wound healing [6,42–44], phenotype manipulation [29], modulation of gene expression [45–50], the antiparasitic effect [51–53], increment of the immune response [54–59], cell proliferation [26–29], improvement in fermentation [60,61] and sterilisation for the food industry [62–64], seed germination [65–67] and, most importantly, cancer treatment [2].

nsPEF-induced damage in cell's DNA

As of May 2022, there is only one study proposing the use of nsPEF as an alternative technology to fight against the COVID-19 pandemic [68]. Given the

CONTACT A. R. Ruiz-Fernández aruiz@dlab.cl Computational Biology Lab, Centro Ciencia & Vida, Fundación Ciencia & Vida, Santiago, Chile

improvement of the immune response after a nsPEF stimulus [54–59], Alawadhi et al. [68] proposed that the delivery of a nsPEF pulse during the application of a SARS-CoV-2 vaccine may increase the title of antibodies, therefore improving the SARS-CoV-2 host immunity. Despite an interesting approach, it is worth mentioning that several studies link the DNA damage caused by nsPEF to cell apoptosis. In fact, DNA damage can be described as a secondary effect of nsPEF application [3,19,69]. Notably, Chen and co-workers showed a strong nsPEF effect on the HL-60 cell nucleus [70]. They observed the quenching of acridine orange (a DNA-intercalating dye) fluorescence after the application of a nsPEF protocol, suggesting that nsPEF directly alters DNA conformation. Stacey et al. [71] registered nsPEF-derived DNA damage by performing a comet assay to evaluate cell survival. To determine if DNA damage was a direct effect of nsPEF, Jurkat cells were lysed and their DNA extracted right after the application of a nsPEF protocol. Their results were interpreted as DNA damage resulting directly from nsPEF exposure [71]. In a continuation study, Stacey et al. [72] collected cells under alkaline conditions to unwind DNA, allowing detection of double- and single-stranded breaks. In their report (see Figure 1) they show a clear DNA fragmentation of nsPEF exposed cells vs the control condition. This direct damage to DNA caused by nsPEF can be understood because DNA is a heavily-charged and polyelectrolyte macromolecule so the proximity of its linear/folded structures to the nuclear membrane might render it susceptible to nsPEF effects. Other studies also support that nsPEF and other closely-related technologies, such as intense burst sinusoidal electric field (IBSEF), can directly cause DNA damage [72–74]. Furthermore, nsPEF exposed cells show a DNA electrophoresis migration profile similar to that observed in gamma-irradiated K562 erythroid cells [75].

Figure 1. Gel electrophoresis of DNA extracted from Jurkat cells right after the application of a nsPEF protocol (60 kV/cm, 60 ns, 5 pulses). Figure 3 extracted from the article "Differential effects in cells exposed to ultra-short, high-intensity electric fields: cell survival, DNA damage, and cell cycle analysis", journal Mutation Research/Genetic Toxicology and Environmental Mutagenesis Volume 542, Issues 1–2, 9 December 2003, Pages 65–75. Reproduced with permission.

Is electroporation an alternative to induce direct DNA damage in cells?

Significant damage to DNA can also be achieved by electroporation (EP) resulting in the activation of apoptotic pathways leading to DNA fragmentation. For instance, irreversible electroporation (IRE) induces DNA fragmentation by apoptosis as determined by TUNEL (terminal deoxynucleotidyl transferase dUTP nick-end labelling) assay [76–78]. Importantly, no available evidence in the literature suggests that EP may induce damage directly to DNA. Thus, we may wonder why nsPEF can damage DNA directly but not EP? This is particularly appealing due to the fact that the electric fields applied in both nsPEF and EP are similar (0.1–100kV/cm) [79]. A theoretical analysis could shed some light on the controversy surrounding this question. If the cell is considered, for the sake of simplicity, like solid metal and conducting sphere, we know that when an external electric field is applied to it, electrons contained in the sphere should migrate to the anode. After a defined amount of time, this continuous migration of electrons should result in an asymmetric charge distribution—creating a self-induced electric field around the sphere (the reaction field) that could nullify the external electric field—resulting in zero electric fields inside the sphere. This is similar to what may happen in cells due to nsPEF application; however, instead of electrons moving around creating an equilibrium in charge distribution, a much longer time, far behind the time-scale of nsPEF application is needed, to nullify the applied electric field. The characteristic time it takes for the external electric field in cells to dissipate is in the order of microseconds or even milliseconds [80]. However, when nsPEF is applied, the pulse duration is in the nanosecond or even sub-nanosecond scale. Hence, during the application of nsPEF, internal charges will continue to move by the influence of the external electric field, continuously perturbing the internal structure and dynamics of the cell.

Conformational changes in proteins due to nsPEF

Besides the formation of nanopores in membranes [4,6,8], extensive available evidence suggests that the application of nsPEF protocols may also affect the

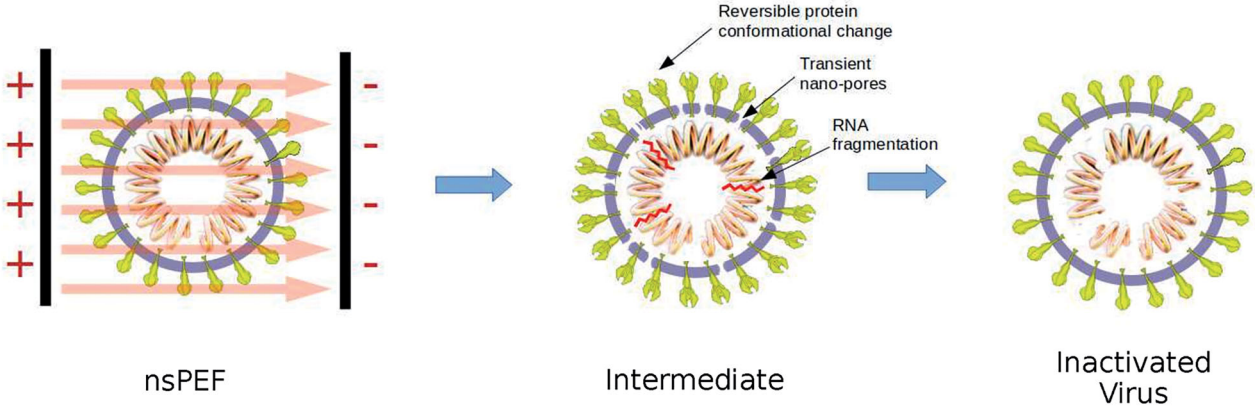

Figure 2. Representation of a nsPEF over a SARS-CoV-2 virus, nsPEF would fragment the viral RNA, with possible transient membrane nano-pores and reversible protein conformational changes. After a certain time interval, the membrane would reseal, and the proteins would return to their native conformation, leaving an inert virus with its initial capsid intact.

structure of voltaged gated (VG) ion channels such as VG Calcium Channels [7,9,10,12–14] and VG Sodium Channels [5,7,11]. Whether nsPEF induces directly the gating of ion channels or their gating is the result of the charge imbalance produced by the movement of ions as a result of the external electric field, is still a matter of debate [81]. Thus, the conformational changes occurring in these channels may either be a direct result of the applied electric field or be the consequence of the internal ion imbalance. Interestingly, available evidence gathered from Molecular Dynamics (MD) simulation suggests that conformational changes resulting from nsPEF occur directly in the voltage sensing structure of the human VG calcium channel [82]. On top of that, Beebe et al. [83] showed that a single nsPEF pulse (the same nsPEF protocol used to elicit DNA damage), decreased the activity of the C-subunit of the cAMP-dependent protein kinase (cAMP-dPKA), during 15 min after nsPEF application. Although the actual mechanisms of nsPEF-induced in-activation remain to be determined, it should happen through reversible conformational changes in a fraction of the cAMP-dPKA proteins: since 15 min after the pulse, the basal activity was recovered. Moreover, particularly interesting for our hypothesis, pulsed electric field technology has been evaluated to determine its ability for viral inactivation. Mizuno el at. [84], used wine vesicular disease virus (SVDV) and equine herpes virus-1 (EHV-1) to determine the effect of high voltage pulsed electric fields. Of note, both viruses were successfully inactivated. Notably, the shape of the protein shell of SVDV remained unaltered while its RNA completely disappeared. Despite apparent damage to the envelope around EHV-1 being detected, the authors suggested that it may be related to either the used medium or some other differences in the applied

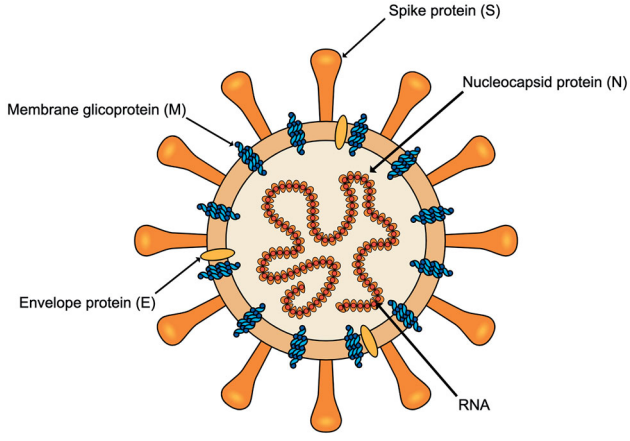

Figure 3. Schematic representation of the SARS-CoV-2 virus showing its RNA and main proteins: spike (S), envelope (E), nucleocapsid (N) and membrane glycoprotein (M).

protocol. It is worth mentioning that MD studies (mimicking a nsPEF protocol consisting of two pulses lasting for 8 ns of 1,000 kV/cm and 10,000 kV/cm) suggested an irreversible conformational change resulting in the disruption of the myoglobin secondary structures without fragmentation [85,86]. However, these MD studies used a much higher electric field compared to that of the 60 kV/cm necessary to produce direct DNA fragmentation in cells.

The application of nsPEF protocols to inactivate SARS-CoV-2

A thorough revision of the literature suggests that a single nsPEF pulse of 60 kV/cm with a duration of 60 ns is actually capable of fragmenting the DNA shielded by the cell nucleus [71]. It is then possible that by modulating different parameters of the nsPEF protocol (i.e. electric field intensity, number of pulses and their duration), a specific combination of them

may fragment SARS-CoV-2 RNA, without causing irreversible conformational changes on the envelop and spike viral proteins. As the SARS-CoV-2 virus has a non-segmented positive single-stranded long RNA, with 26–32 kb [87,88], it is expected to be more susceptible to nsPEF-derived single-stranded breaks as the energy required to produce double-stranded breaks is much higher. Therefore, in our opinion, the application of a specific nsPEF protocol into a solution containing SARS-CoV-2 viral particles may result in RNA fragmentation and a completely inert viral particle (Figure 2). Importantly, if the elicited conformational changes occurring in viral proteins are indeed reversible, it will overcome one of the main problems of current vaccines using inactivated viruses. Traditional methods to produce inactivated viruses use heat, detergents and other chemicals. These are aggressive techniques that may produce drastic changes in the conformation of proteins, particularly in the case of spike proteins in SARS-CoV-2. These conformational changes may occlude or even create new epitopes that can hinder the immune response. Of note, Liu et al. used state-of-the-art cryo-electron microscopy technologies to characterize the architecture of inactivated SARS-CoV-2 viruses [89]. The authors found that the viral spikes are mostly in a postfusion state, a conformation that is not desirable for vaccine development because, *in vivo*, neutralising antibodies will not recognise the prefusion conformation of the SARS-CoV-2 spike (S) protein. Additionally, there is no evidence of whether the rest of the SARS-CoV-2 proteins retain their conformation after inactivation by current methods. Thus, maintaining the native conformation is particularly interesting as the majority of RNA-based vaccines only encode for the target protein antigen of the SARS-CoV-2 virus. In fact, four of the most used vaccines worldwide [90] are RNA-based encoding for the S protein [91]. The usage of an inactivated virus having its proteins in its biologically relevant conformation may elicit a more relevant immune response. Therefore, other SARS-CoV-2 proteins exposed in the capsid besides the S protein such as the envelope (E), nucleocapsid (N) and membrane glycoprotein (M) may also play a role in the enhancement of an appropriate immune response (Figure 3) [92].

As was described in the introduction, one of the primary effects of nsPEF is the induction of nanopores in cell membranes [4,6,8]. Although these nanopores could also appear in the SARS-CoV-2 membrane after the application of a nsPEF protocol they would spontaneously reseal after the given time. Evidence for this resealing of the pores has been reported in EP. Saulis et al. [93] observed the complete resealing of membrane pores occurring in human red blood cells, 20–30 min after the application of an EP electric pulse of 4 kV/cm of 2μs. This transient formation of pores has also been reported in other cell types [94–96]. In the case of nanopores, the resealing process is much faster. Recent results conclude that nanopore resealing occurs in a couple of minutes [97–103].

It is important to mention that other kinds of electromagnetic waves capable to damage nucleic acids, such as x-Ray [104] or gamma-Radiation [105] based on highly unspecific ionising radiations are not suitable for viral inactivation, since their photons are also absorbed by proteins. Thus, these radiations cannot produce inactivated SARS-CoV-2 viruses suitable to be used for a vaccine because their application will elicit irreversible conformational changes in the SARS-CoV-2 proteins. Therefore, the advantage of nsPEF is that it may be a suitable technique than can be used for the production of highly immunogenic and inactivated SARS-CoV-2 viruses.

Despite seeming an obvious alternative, we believe that nsPEF has not been taken into account perhaps because it is a new field that most molecular biologists are unfamiliar with. Additionally, this technique may be of particular interest to the industry considering that nsPEF is an inexpensive and accessible technology. In fact, it is perfectly feasible to build an in-house nsPEF device. An excellent review remarking on this point can be read at [106].

Conclusions

A larger body of research is needed to capitalise on the basic knowledge accumulated in the nsPEF field in order to translate it into real demonstrable applications. This paper proposes one of such new applications. In the light of the continuous appearance of new SARS-CoV-2 variants, evaluating nsPEF to generate inactivated SARS-CoV-2 that may, in turn, be used for the production of novel vaccines, is an urgent task. It is expected that, after the application of a suitable nsPEF protocol an inactivated virus harbouring intact proteins and fragmented RNA, should be produced. Thus, resulting inactivated viruses could be used to develop a novel SARS-CoV-2 vaccine where inert viruses expose their proteins in the same conformation as the original viruses. This will ensure that the viral epitopes will remain intact boosting the immune response. This new approach to vaccine development may be not only important to fight against the

COVID-19 pandemic, but also to develop vaccines suitable to better address future pandemics.

Acknowledgments

The authors acknowledge to Dr. Andrés Hojman for his suggestions to improve this article.

Author contributions

Conceptualisation, writing original draft preparation A.R.; Writing review and editing, supervision, M.R.; Writing review and editing, funding acquisition, project administration T.P.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The authors are pleased to acknowledge financial support from FONDECYT Iniciación 11221268 and from Cen-tro Ciencia & Vida, FB210008, Programa de Financiamiento Basal para Centros Cientificos y Tecnológicos de Excelencia de ANID.

References

[1] Schoenbach KH, Alden RW, Fox TJ. Biofouling prevention with pulsed electric fields. In: Proceedings of 1996 International Power Modulator Symposium. IEEE; 1996, p. 75–78. doi:10.1109/MODSYM.1996.564454.

[2] Batista Napotnik T, Reberšek M, Vernier PT, et al. Effects of high voltage nanosecond electric pulses on eukaryotic cells (in vitro): a systematic review. Bioelectro- Chem. 2016;110:1–12.

[3] Beebe SJ, Fox PM, Rec LJ, et al. Nanosecond Pulsed Electric Field (nsPEF) effects on cells and tissues: apoptosis induction and tumor growth inhibition. IEEE Trans Plasma Sci. 2002;30(1):286–292.

[4] Beebe SJ, Fox PM, Rec LJ, et al. Nanosecond, high-intensity pulsed electric fields induce apoptosis in human cells. Faseb J. 2003;17(11):1493–1495.

[5] Rogers WR, Merritt JH, Comeaux JA, et al. Strength-duration curve for an electrically excitable tissue extended down to near 1 nanosecond. IEEE Trans Plasma Sci. 2004;32(4):1587–1599.

[6] Zhang J, Blackmore PF, Hargrave BY, et al. Nanosecond pulse electric field (nanopulse): a novel non-ligand agonist for platelet activation. Arch Biochem Biophys. 2008;471(2):240–248.

[7] Craviso GL, Choe S, Chatterjee P, et al. Nanosecond electric pulses: a novel stimulus for triggering Ca2+ influx into chromaffin cells via voltage-gated Ca2+ channels . Cell Mol Neurobiol. 2010;30(8):1259–1265.

[8] Semenov I, Xiao S, Pakhomov AG. Primary pathways of intracellular ca 2+ mobilization by nanosecond pulsed electric field. Biochim Biophys Acta (BBA)-Biomembranes. 2013;1828(3):981–989.

[9] Semenov I, Xiao S, Kang D, et al. Cell stimulation and calcium mobilization by picosecond electric pulses. Bioelectrochemistry. 2015;105:65–71.

[10] Burke RC, Bardet SM, Carr L, et al. Nanosecond pulsed electric fields depolarize transmembrane potential via voltage- gated k+, ca2+ and trpm8 channels in u87 glioblastoma cells. Biochim Biophys Acta (BBA)- Biomembranes. 2017;1859(10):2040–2050.

[11] Pakhomov AG, Semenov I, Casciola M, et al. Neuronal excitation and permeabilization by 200-ns pulsed electric field: an optical membrane potential study with fluovolt dye. Biochim Biophys Acta (BBA)-Biomembranes. 2017;1859(7):1273–1281.

[12] Bagalkot TR, Terhune RC, Leblanc N, et al. Different membrane path- ways mediate ca2+ influx in adrenal chromaffin cells exposed to 150–400 ns electric pulses. Biomed Res Int. 2018;2018:1–12.

[13] Hristov K, Mangalanathan U, Casciola M, et al. Expression of voltage-gated calcium channels augments cell susceptibility to membrane disruption by nanosecond pulsed electric field. Biochim Biophys Acta (BBA)-Biomembranes. 2018;1860(11):2175–2183.

[14] Bagalkot TR, Leblanc N, Craviso GL. Stimulation or cancellation of ca 2+ influx by bipolar nanosecond pulsed electric fields in adrenal chromaffin cells can be achieved by tuning pulse waveform. Sci Rep. 2019;9(1):1–13.

[15] White JA, Blackmore PF, Schoenbach KH, et al. Stimulation of capacitative calcium entry in hl-60 cells by nanosecond pulsed electric fields. J Biol Chem. 2004;279(22):22964–22972.

[16] Pakhomova ON, Gregory B, Semenov I, et al. Calcium-mediated pore expansion and cell death following nanoelectroporation. Biochim Biophys Acta (BBA)-Biomembranes. 2014;1838(10):2547–2554.

[17] Zhou P, He F, Han Y, et al. Nanosecond pulsed electric field induces calcium mobilization in osteoblasts. Bioelectrochemistry. 2018;124:7–12.

[18] Carr L, Bardet SM, Arnaud-Cormos D, et al. Visualisation of an nsPEF induced calcium wave using the genetically encoded calcium indicator GCAMP in U87human glioblastoma cells. Bioelectrochemistry. 2018;119:68–75.

[19] Beebe SJ, Fox PM, Rec LJ, et al. Nanosecond pulsed electric field (nsPEF) effects on cells and tissues: apoptosis induction and tumor growth inhibition. IEEE Trans Plasma Sci. 2002;30(1):286–292. doi:10.1109/TPS.2002.1003872.

[20] Hall EH, Schoenbach KH, Beebe SJ. Nanosecond pulsed electric fields induce apoptosis in p53-wild-type and p53-null HCT116 colon carcinoma cells. Apoptosis. 2007;12(9):1721–1731.

[21] Ford WE, Ren W, Blackmore PF, et al. Nanosecond pulsed electric fields stimulate apoptosis without release of pro-apoptotic factors from mitochondria in B16f10 melanoma. Arch Biochem Biophys. 2010;497(1–2):82–89.

[22] Chen X, Kolb JF, Swanson RJ, et al. Apoptosis initiation and angiogenesis inhibition: melanoma targets for nanosecond pulsed electric fields. Pigment Cell Melanoma Res. 2010;23(4):554–563.
[23] Ren W, Beebe SJ. An apoptosis targeted stimulus with nanosecond pulsed electric fields (nsPEFs) in e4 squamous cell carcinoma. Apoptosis. 2011;16(4): 382–393.
[24] Beebe SJ, Sain NM, Ren W. Induction of cell death mechanisms and apoptosis by nanosecond pulsed electric fields (nsPEFs). Cells. 2013;2(1):136–162.
[25] He L, Xiao D, Feng J, et al. Induction of apoptosis of liver cancer cells by nanosecond pulsed electric fields (nsPEFs). Med Oncol. 2017;34(2):24.
[26] Dong F, Liu Z, Zhang J, et al. nsPEFs promoting the proliferation of piec cells: an in vitro study. In 2017 IEEE International Conference on Plasma Science (ICOPS). IEEE; 2017, p. 1–1. doi:10.1109/PLASMA.2017.8496052.
[27] Zhang Y, Dong F, Liu Z, et al. Nanosecond pulsed electric fields promoting the proliferation of porcine iliac endothelial cells: an in vitro study. PLOS One. 2018;13(5):e0196688.
[28] Buchmann L, Frey W, Gusbeth C, et al. Effect of nanosecond pulsed electric field treatment on cell proliferation of microalgae. Bioresour Technol. 2019; 271:402–408.
[29] Vadlamani RA, Nie Y, Detwiler DA, et al. Nanosecond pulsed electric field induced proliferation and differentiation of osteoblasts and myoblasts. J R Soc Interface. 2019;16(155):20190079.
[30] Schoenbach KH, Abou-Ghazala A, Vithoulkas T, et al. The effect of pulsed electrical fields on biological cells. In: Digest of Technical Papers. 11th IEEE International Pulsed Power Conference (Cat. No. 97CH36127), Vol. 1. IEEE; 1997, p. 73–78. doi:10.1109/PPC.1997.679279.
[31] Müller KJ, Sukhorukov VL, Zimmermann U. Reversible electropermeabilization of mammalian cells by high-intensity, ultra-short pulses of submicrosecond duration. J Membr Biol. 2001;184(2): 161–170.
[32] Schoenbach KH, Beebe SJ, Buescher ES. Intracellular effect of ultrashort electrical pulses. Bioelectromagnetics. 2001; 22(6):440–448.
[33] Gowrishankar TR, Esser AT, Vasilkoski Z, et al. Microdosimetry for conventional and supra-electroporation in cells with organelles. Biochem Biophys Res Commun. 2006;341(4):1266–1276.
[34] Roth CC, Tolstykh GP, Payne JA, et al. Nanosecond pulsed electric field thresholds for nanopore formation in neural cells. J Biomed Opt. 2013;18(3):035005.
[35] Romanenko S, Arnaud-Cormos D, Leveque P, et al. Ultrashort pulsed electric fields induce action potentials in neurons when applied at axon bundles. In: 2016 9th International Kharkiv symposium on physics and engineering of microwaves, Millimeter and Submillimeter Waves (MSMW). IEEE; 2016, p. 1–5.
[36] Casciola M, Xiao S, Pakhomov AG. Damage-free peripheral nerve stimulation by 12-ns pulsed electric field. Sci Rep. 2017;7(1):1–8.
[37] Lamberti P, Tucci V, Zeni O. Stefania Romeo. Analysis of ionic channel currents under nsPEFs-stimulation by a circuital model of an excitable cell. In. 2020 IEEE 20th Mediterranean Electrotechnical Conference (MELECON). IEEE; 2020, p. 411–414.
[38] Wang S, Chen J, Chen M-T, et al. Cardiac myocyte excitation by ultrashort high-field pulses. Biophys J. 2009;96(4):1640–1648.
[39] Semenov I, Grigoryev S, Neuber JU, et al. Excitation and injury of adult ventricular cardiomyocytes by nano-to millisecond electric shocks. Sci Rep. 2018; 8(1):1–12.
[40] Azarov JE, Semenov I, Casciola M, et al. Excitation of murine cardiac myocytes by nanosecond pulsed electric field. J Cardiovasc Electrophysiol. 2019;30(3): 392–401.
[41] Pakhomov AG, Xiao S, Novickij V, et al. Excitation and electroporation by MHz bursts of nanosecond stimuli. Biochem Biophys Res Commun. 2019;518(4): 759–764.
[42] Xiao S, Kiyan T, Blackmore PF, et al. Pulsed power for wound healing. In: 2008 IEEE international power modulators and high-voltage conference. IEEE; 2008, p. 69–72.
[43] Hargrave B, Li F. Nanosecond pulse electric field activation of platelet-rich plasma reduces myocardial infarct size and improves left ventricular mechanical function in the rabbit heart. J Extra Corpor Technol. 2012;44(4):198–204.
[44] Hargrave B, Li F. Nanosecond pulse electric field activated-platelet rich plasma enhances the return of blood flow to large and ischemic wounds in a rabbit model. Physiol Rep. 2015;3(7):e12461.
[45] Beebe SJ, Blackmore PF, White J, et al. Nanosecond pulsed electric fields modulate cell function through intracellular signal transduction mechanisms. Physiol Meas. 2004;25(4):1077–1093.
[46] Morotomi-Yano K, Uemura Y, Katsuki S, et al. Activation of the JNK pathway by nanosecond pulsed electric fields. Biochem Biophys Res Commun. 2011;408(3):471–476.
[47] Zhang K, Guo J, Ge Z, et al. Nanosecond pulsed electric fields (nsPEFs) regulate phenotypes of chondrocytes through Wnt/β -catenin signaling pathway. Sci Rep. 2014;4:5836.
[48] Guo S, Jackson DL, Burcus NI, et al. Gene electrotransfer enhanced by nanosecond pulsed electric fields. Mol Ther Methods Clin Dev. 2014;1:14043.
[49] Estlack LE, Roth CC, Thompson GL, et al. Nanosecond pulsed electric fields modulate the expression of Fas/CD95 death receptor pathway regulators in U937 and Jurkat cells. Apoptosis. 2014; 19(12):1755–1768.
[50] Muratori C, Pakhomov AG, Gianulis E, et al. Activation of the phospholipid scramblase tmem16f by nanosecond pulsed electric fields (nsPEF) facilitates its diverse cytophysiological effects. J Biol Chem. 2017;292(47):19381–19391.
[51] Zhang R, Aji T, Shao Y, et al. Nanosecond pulsed electric field (nsPEF) disrupts the structure and metabolism of human echinococcus granulosus

protoscolex in vitro with a dose effect. Parasitol Res. 2017;116(4):1345–1351.
[52] Chen X, Zhang R, Aji T, et al. Novel interventional management of hepatic hydatid cyst with nanosecond pulses on experimental mouse model. Sci Rep. 2017;7(1):1–8.
[53] Chen X, Zhang R, Wen H. Experimental nanopulse ablation of multiple membrane parasite on ex vivo hydatid cyst. Biomed Res Int. 2018;2018:1–9.
[54] Nuccitelli R, Tran K, Lui K, et al. Non-thermal nanoelectroablation of UV-induced murine melanomas stimulates an immune response. Pigment Cell Melanoma Res. 2012;25(5):618–629.
[55] Chen R, Sain NM, Harlow KT, et al. A protective effect after clearance of orthotopic rat hepatocellular carcinoma by nanosecond pulsed electric fields. Eur J Cancer. 2014;50(15):2705–2713.
[56] Nuccitelli R, Berridge JC, Mallon Z, et al. Nanoelectroablation of murine tumors triggers a CD8-dependent inhibition of secondary tumor growth. PLOS One. 2015;10(7):e0134364.
[57] Nuccitelli R, McDaniel A, Anand S, et al. Nano-pulse stimulation is a physical modality that can trigger immunogenic tumor cell death. J Immunother Cancer. 2017;5(1):32.
[58] Guo S, Jing Y, Burcus NI, et al. Nano-pulse stimulation induces potent immune responses, eradicating local breast cancer while reducing distant metastases. Int J Cancer. 2018;142(3):629–640.
[59] Skeate JG, Da Silva DM, Chavez-Juan E, et al. Nanopulse stimulation induces immunogenic cell death in human papillomavirus-transformed tumors and initiates an adaptive immune response. PLOS One. 2018;13(1):e0191311.
[60] Guo J, Ma R, Su B, et al. Raising the avermectins production in Streptomyces avermitilis by utilizing nanosecond pulsed electric fields (nsPEFs). Sci Rep. 2016;6(1):25949.
[61] Rajabi F, Gusbeth C, Frey W, et al. Nanosecond pulsed electrical fields enhance product recovery in plant cell fermentation. Protoplasma. 2020;257(6):1585–1510.
[62] Prorot A, Arnaud-Cormos D, Lévêque P, et al. Bacterial stress induced by nanosecond pulsed electric fields (nsPEF): potential applications for food industry and environment. In IV International Conference on Environmental, Industrial and Applied Microbiology, 2011 Sep 14, p. 194.
[63] Buchmann L, Böcker L, Frey W, et al. Energy input assessment for nanosecond pulsed electric field processing and its application in a case study with chlorella vulgaris. Innov Food Sci Emerg Technol. 2018;47:445–453.
[64] Haberkorn I, Buchmann L, Häusermann I, et al. Nanosecond pulsed electric field processing of microalgae based biorefineries governs growth promotion or selective inactivation based on underlying microbial ecosystems. Bioresour Technol. 2021;319:124173.
[65] Eing CJ, Bonnet S, Pacher M, et al. Effects of nanosecond pulsed electric field exposure on Arabidopsis thaliana. IEEE Trans Dielect Electr Insul. 2009;16(5):1322–1328.
[66] Songnuan W, Kirawanich P. Early growth effects on Arabidopsis thaliana by seed exposure of nanosecond pulsed electric field. J Electrostat. 2012;70(5):445–450.
[67] Su B, Guo J, Nian W, et al. Early growth effects of nanosecond pulsed electric field (nsPEFs) exposure on Haloxylon ammodendron. Plasma Process Polym. 2015;12(4):372–379.
[68] Allawadhi P, Khurana A, Allwadhi S, et al. Potential of electric stimulation for the management of covid-19. Med Hypotheses. 2020;144:110259.
[69] Kim HB, Ahn S, Sim SB. Apoptosis by direct electric field (def) and nanosecond pulsed electric field (nsPEF) in tumor cells and tumor tissues. In: The 30th International Conference on Plasma Science, 2003. ICOPS 2003. IEEE Conference Record-Abstracts. IEEE, 2003, p. 436.
[70] Chen N, Schoenbach KH, Kolb JF, et al. Leukemic cell intracellular responses to nanosecond electric fields. Biochem Biophys Res Commun. 2004;317(2):421–427.
[71] Stacey M, Stickley J, Fox P, et al. Increased cell killing and DNA damage in cells exposed to ultra-short pulsed electric fields. In: Electrical Insulation and Dielectric Phenomena, 2002 Annual Report Conference on. IEEE, 2002, p. 79–82.
[72] Stacey M, Stickley J, Fox P, et al. Differential effects in cells exposed to ultra-short, high intensity electric fields: cell survival, DNA damage, and cell cycle analysis. Mutat Res Genet Toxicol Environ Mutagen. 2003;542(1–2):65–75.
[73] Beebe SJ, White J, Blackmore PF, et al. Diverse effects of nanosecond pulsed electric fields on cells and tissues. DNA Cell Biol. 2003;22(12):785–796.
[74] Nomura N, Yano M, Katsuki S, et al. Intracellular DNA damage induced by non-thermal, intense narrowband electric fields. IEEE Trans Dielect Electr Insul. 2009;16(5):1288–1293.
[75] Tabocchini M-A, Rothkamm K, Signoretti C, et al. Formation and repair of DNA double-strand breaks in γ-irradiated k562 cells undergoing erythroid differentiation. Mutat Res. 2000;461(1):71–82.
[76] Kim H-B, Sung C-K, Baik KY, et al. Changes of apoptosis in tumor tissues with time after irreversible electroporation. Biochem Biophys Res Commun. 2013;435(4):651–656.
[77] Lee JM, Choi HS, Kim ES, et al. Characterization of irreversible electroporation on the stomach: a feasibility study in rats. Sci Rep. 2019;9(1):1–11.
[78] Long G, Bakos G, Shires PK, et al. Histological and finite element analysis of cell death due to irreversible electroporation. Technol Cancer Res Treat. 2014;13(6):561–569.
[79] Weaver JC, Smith KC, Esser AT, et al. A brief overview of electroporation pulse strength-duration space: a region where additional intracellular effects are expected. Bioelectrochemistry. 2012;87:236–243.
[80] Koch C, Rapp M, Segev I. A brief history of time (constants). Cereb Cortex. 1996;6(2):93–101.

[81] Rems L, Kasimova MA, Testa I, et al. Pulsed electric fields can create pores in the voltage sensors of voltage-gated ion channels. Biophys J. 2020;119(1): 190–205.
[82] Ruiz-Fernández AR, Campos L, Villanelo F, et al. Exploring the conformational changes induced by nanosecond pulsed electric fields on the voltage sensing domain of a CA2+ channel. Membranes. 2021;11(7):473.
[83] Beebe SJ. Considering effects of nanosecond pulsed electric fields on proteins. Bioelectrochemistry. 2015; 103:52–59.
[84] Mizuno A, Inoue T, Yamaguchi S, et al. Inactivation of viruses using pulsed high electric field. In: Conference Record of the 1990 IEEE Industry Applications Society Annual Meeting. IEEE; 1990, p. 713–719.
[85] Marracino P, Apollonio F, Liberti M, et al. Effect of high exogenous electric pulses on protein conformation: myoglobin as a case study. J Phys Chem B. 2013;117(8):2273–2279.
[86] Marracino P, Paffi A, Reale R, et al. Technology of high-intensity electric-field pulses: a way to control protein unfolding. J Phys Chem Biophys. 2013;3(02): 2161–0398.
[87] Velavan TP, Meyer CG. The covid-19 epidemic. Trop Med Int Health. 2020;25(3):278–280.
[88] Elfiky AA. SARS-CoV-2 RNA dependent RNA polymerase (RdRp) targeting: an in silico perspective. J Biomol Struct Dyn. 2021;39(9):3204–3212.
[89] Liu C, Mendonça L, Yang Y, et al. The architecture of inactivated SARS-CoV-2 with postfusion spikes revealed by Cryo-EM and Cryo-ET. Structure. 2020; 28(11):1218–1224.
[90] Tracking Coronavirus Vaccinations Around the World; 2022. Retrieved from https://www.nytimes.com/interactive/2021/world/covid-vaccinations-tracker.html.
[91] Thanh Le T, Andreadakis Z, Kumar A, et al. The covid-19 vaccine development landscape. Nat Rev Drug Discov. 2020;19(5):305–306.
[92] Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. In: Maier H, Bickerton E, Britton P, editors. Coronaviruses. Methods in Molecular Biology, vol 1282. New York, NY: Humana Press. doi:10.1007/978-1-4939-2438-7_1.
[93] Saulis G, Venslauskas MS, Naktinis J. Kinetics of pore resealing in cell membranes after electroporation. J Electroanal Chem Interfacial Electrochem. 1991; 321(1):1–13.
[94] Zimmermann U, Vienken J, Pilwat G. Development of drug carrier systems: electrical field induced effects in cell membranes. J Electroanal Chem Interfacial Electrochem. 1980;116:553–574.
[95] Escande-Géraud ML, Rols MP, Dupont MA, et al. Reversible plasma membrane ultrastructural changes correlated with electropermeabilization in Chinese hamster ovary cells. Biochim Biophys Acta. 1988; 939(2):247–259.
[96] Kinosita K, Ashikawa I, Saita N, et al. Electroporation of cell membrane visualized under a pulsed-laser fluorescence microscope. Biophys J. 1988;53(6): 1015–1019.
[97] Pakhomov AG, Kolb JF, White JA, et al. Long-lasting plasma membrane permeabilization in mammalian cells by nanosecond pulsed electric field (nsPEF). Bioelectromagnetics. 2007;28(8):655–663.
[98] Vernier PT, Sun Y, Chen M-T, et al. Nanosecond electric pulse-induced calcium entry into chromaffin cells. Bioelectrochemistry. 2008;73(1):1–4.
[99] Pakhomov AG, Bowman AM, Ibey BL, et al. Lipid nanopores can form a stable, ion channel-like conduction pathway in cell membrane. Biochem Biophys Res Commun. 2009;385(2):181–186.
[100] Pakhomov AG, Miklavcic D, Markov MS. Advanced electroporation techniques in biology and medicine. CRC Press, 2010.
[101] Pakhomov AG, Pakhomova ON. Nanopores: a distinct transmembrane passageway in electroporated cells. In: Advanced electroporation techniques in biology in medicine. 2010; p. 178–194.
[102] Bowman AM, Nesin OM, Pakhomova ON, et al. Analysis of plasma membrane integrity by fluorescent detection of TL+ uptake. J Membrane Biol. 2010;236(1):15–26.
[103] Ibey BL, Pakhomov AG, Gregory BW, et al. Selective cytotoxicity of intense nanosecond-duration electric pulses in mammalian cells. Biochim Biophys Acta (BBA)-General Subjects. 2010;1800(11):1210–1219.
[104] Rothkamm K, Löbrich M. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low X-ray doses. Proc Natl Acad Sci USA. 2003;100(9):5057–5062.
[105] Sudprasert W, Navasumrit P, Ruchirawat M. Effects of low-dose gamma radiation on DNA damage, chromosomal aberration and expression of repair genes in human blood cells. Int J Hyg Environ Health. 2006;209(6):503–511.
[106] Butkus P, Murauskas A, Tolvaišienė S, et al. Concepts and capabilities of in-house built nanosecond pulsed electric field (nsPEF) generators for electroporation: state of art. Appl Sci. 2020;10(12):4244.