# Nanosecond Pulsed Electric Field Applications

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Nanosecond Pulsed Electric Field (nsPEF) is an electrostimulation technique first developed in 1995; nsPEF requires the delivery of a series of pulses of high electric fields in the order of nanoseconds into biological tissues or cells. They primary effects in cells is the formation of membrane nanopores and the activation of ionic channels, leading to an incremental increase in cytoplasmic Ca2+ concentration, which triggers a signaling cascade producing a variety of effects: from apoptosis up to cell differentiation and proliferation. Further, nsPEF may affect organelles, making nsPEF a unique tool to manipulate and study cells. This technique is exploited in a broad spectrum of applications, such as: sterilization in the food industry, seed germination, anti-parasitic effects, wound healing, increased immune response, activation of neurons and myocites, cell proliferation, cellular phenotype manipulation, modulation of gene expression, and as a novel cancer treatment.

nsPEF NPS nanopores ionic channels medical devices cancer

## 1. A Brief History on the Development of Electric Pulses Technology

The use of electricity in humans can be traced back to the 18th century, when tissue damage was observed after the application of electric fields [1]. Despite the occurrence of lesions on the skin of humans and animals after exposure to electric sparks, the mechanism of action was far from being understood. Much latter, circa 1982, Neumann et al. achieved the first DNA transfection into cells [2] by applying a protocol including an electric field of 8 kV/cm for 5 µs, inducing a phenomenon in the cell membrane they termed *electroporation*. Almost a decade later, Pakhomov et al. demonstrated that the application of electric fields on cells creates water-filled lipid nanopores forming a stable, ion channel-like conduction pathway in the cell membrane [3]. Denoting its appropriateness, the definition of electroporation has remained intact for over 30 years: “electroporation is the transient loss of semi-permeability of cell membranes under the application of electric pulses, leading to ion leakage, the escape of metabolites, and increased cell-uptake of drugs, molecular probes, and DNA” [4]. Since its remote origins, this technology is nowadays widely used for several applications other than DNA transfection, such as electrochemotherapy [5][6], tissue ablation [7][8], extraction of chemical compounds [9][10], and microbial inactivation for food preservation [11], among others. The next significant step along the historical evolution of the application of electric pulses to biological systems occurred in 1995, when Schoenbach et al. developed a technique to generate high intensity nano-pulsed electric fields, on the order of 6.45 kV/cm with a duration ~700 ns, to treat natural water

used in industrial cooling systems [12]. This technique is nowadays known by the academic community either as nanosecond Pulsed Electric Field (nsPEF) or Nano Pulse Stimulation (NPS). Later on, Schoenbach started a longstanding collaboration with Stephen J. Beebe; together they pioneered the nsPEF field, studying systematically its effects in cells through both theoretical and experimental approaches, giving this technique a new spectrum of applicability. By stepping into the sub-nanosecond realm, inspired by a note from Carl E. Baum in 2005 and later published in 2007 [13], Heeren et al. used an impulse radiating antenna (IRA) instead of electrodes to deliver an electric pulse with a peak amplitude of about 250 kV and with a pulse-width of ~600 ps [14]. This development added two main advantages to the field: the capability of delivering an electric pulse in the order of picoseconds, and the ability to target deeper body tissues, allowing the application of nsPEF in vivo. **Figure 1** summarizes the main events through time in the development of nsPEF technology.

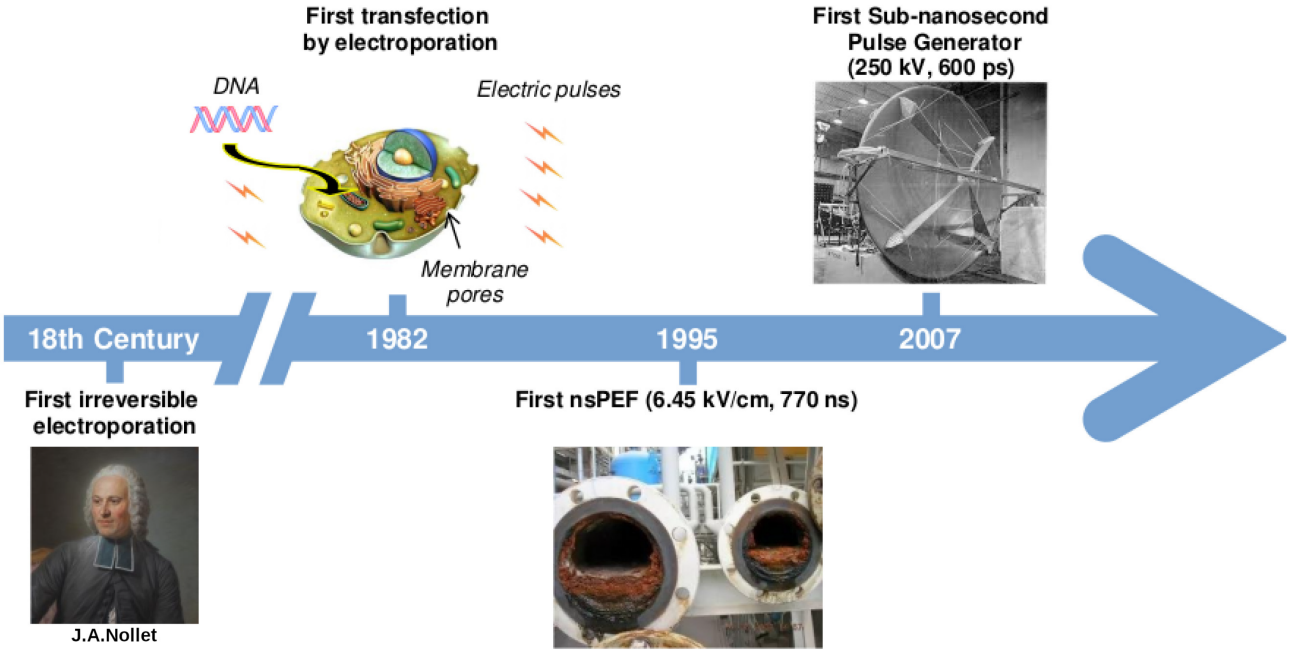


**Figure 1.** Timeline of main events in the development of electric pulse technology. The first application of electric pulses was recorded in 1754 with the experiments performed by J. A. Nollet. Two centuries later, in 1982, E. Neumann et al. [2] coined the term electroporation to describe the use of electric pulses to create membrane pores allowing the insertion of genetic material into cells. Afterwards, in 1995, Schoenbach et al. [12] developed the first nsPEF technology to prevent biofouling of cooling systems. Lately, the construction of an IRA in 2007 by Heeren et al. [13], allowed the application of sub-nanosecond pulses.

# 2. nsPEF Applications

## 2.1. In Human Health

- **Activation of excitable cells**:

**Cardiac cells**: nsPEF (10–80 kV/cm, 4 ns, 1–20 pulses with 200/400/600 ms intervals) can indirectly lead to cardiac cell excitation. Of note, these results challenge the concept of chronaxie: minimum time required for an electric current to double the strength of the rheobase in order to stimulate a muscle or a neuron. The use of nsPEF technology to excite cardiac cells and mobilize intracellular Ca2+ may prove valuable for cardiac pacing and defibrillation [15]. For other related studies see [16][17][18].

**Neurons**: nsPEF (27.8 kV/cm, 10 ns, single pulse) was sufficient to initiate action potentials. The observed effect was repeatable and stable. These results highlight the potential use of ultrashort pulsed electric fields for stimulation of subcortical structures and suggest they may be used as a wireless alternative for deep brain stimulation [19]. For other related studies see [20][21][22][23].

- **Phenotype manipulation**:

**Differentiation**: nsPEF (1.5–25 kV/cm, 300 ns, 5 pulses) can induce proliferation and myotubule maturation or nodule formation in myoblasts and osteoblasts, respectively. Myoblasts were isolated from hind-limb skeletal muscle of four-week-old mice *Pten*$^{MKO}$, and primary human osteoblasts were obtained from a vendor (Sciencell®) [24].

**Dedifferentiation**: nsPEF (10–20 kV/cm, 100 ns pulse) induces dedifferentiation partially through transient activation of the wnt/*β*–catenin signaling pathway in porcine chondrocytes [25].

- **Gene expression**: nsPEF (20 kVcm, 80 ns, various combinations of pulses) dramatically elevated c-Jun and c-Fos mRNA levels, which correlated with the observation of c-Jun N-terminal kinase (JNK) pathway activation in HeLa S3 [26]. For related studies see [25][27][28][29][30].

- **ntiparasitic**: Cystic echinococcosis is a widely endemic helminthic disease caused by infection with metacestodes (larval stage) of the *Echinococcus granulosus* tapeworm. Application of nsPEF (21 KV/cm, 300 ns, 100 pulses) caused a significant increase in the death rate of protoscolices (future heads of the adult worms) [31]. For related studies see [32][33].

- **Wound healing**: nsPEF (30 kV/cm, 300 ns) induced platelet rich plasma aggregation and platelet gel formation. These gels are applied to soft and hard tissue wounds, where they enhance healing [34]. For other related studies see [35][36][37].

- **Immune response**: Using in vivo experiments, nsPEF (15 kV, 100 ns, 400 pulses) induced translocation of calreticulin in rat tumor cell-surfaces, a molecular pattern associated with damage that is indicative of immunogenic cell death (ICD). The nsPEF also triggered CD8-dependent inhibition of secondary tumor growth, concluded by comparing the tumor size using rats depleted of $CD8^+$cytotoxic T-cells under the same nsPEF treatment. The first group showed an average size of only 3% of the primary tumor size compared with the 54% shown by the $CD8^+$-depleted rats. Additionally, with immunohistochemistry it was observed that CD8+ T-cells were highly enriched in the first group. Furthermore, it was shown that vaccinating rats with isogenic tumor cells (MCA205 fibrosarcoma cell line) treated with nsPEF (50 kV, 100 ns, 500 pulses) stimulates an immune

response that inhibits the growth of secondary tumors in a $CD8^+$-dependent manner [38]. This work opens the door to the fabrication of cell-based vaccines using nsPEF stimulation to promote an improved immune response. For other related studies reporting tumor ablation through an antitumor immune response using nsPEF see [39][40][41][42][43].

- **Cancer**: This is by far the most-studied nsPEF application, with 46 in vitro studies up to 2016 [44] and over 100 so far. Recently, preclinical animal studies have demonstrated that nsPEF can induce local and systemic CD8+T-cell mediated adaptive immune response against tumors [40][43]. In clinical trials, nsPEF proved to be a safe and effective therapy against basal cell carcinoma [45][46]. There are other novel techniques to combat cancer that also use electric fields, known as electrochemotherapy [47][48], irreversible electroporation [7], and electro-gene therapy [7]. Electrochemotherapy and electro-gene therapy use electroporation to achieve the anti-tumoral effect of other agents. In irreversible electroporation, cytoplasmic membranes of tumor cells cannot recover from permeabilization, causing cell death mainly by necrosis. Unlike the just mentioned electro-technique, nsPEF is cell-dependent. A possible explanation for this may be related to apoptosis (programmed cell death type 1 [49]), which is a tightly controlled cell process and different in each cell type [50]. Thus, if nsPEF induces apoptosis, as seems to be the case, it is expected to exhibit cell-dependent responses. This makes nsPEF an extraordinary tool, with specific responses based on tuning the intensity, duration, and number of pulses. There are several examples of cell dependence and nsPEF. Stacey et al. in 2002 demonstrated that exposing cancer cells to nsPEF with 60 kV/cm could induce DNA damage [51]. Beebe et al. in 2002 studied the antitumor effects of nsPEF on Jurkat cells, with pulses at 60, 150, and 300 kV/cm [52]. Xinh ua Chen et al. in 2012 applied nsPEF with 900 pulses at 68 kV/cm to ablate hepatocellular carcinoma [53]. Nuccitelli et al. in 2013 inhibited human pancreatic carcinoma using 100 pulses of 100 ns duration and 30 kV/cm [54]. More importantly for nsPEF as cancer treatment, tumor cells are more sensitive to nsPEF than normal cells [55].

## 2.2. Industrial

- **Cell proliferation**: nsPEF (10 kV/cm, 100 ns) can increase *Arthrospira platensis* SAG 21.99 (a cyanobacteria) cell growth after repeated pulses in the exponential growth phase. The effect was most pronounced five days after treatment. Treatments with nsPEF might improve sustainable and economical microalgae-based biorefineries [57]. For other studies see [24][58][59].

- **Fermentation industry**: nsPEF (15 kV/cm, 100 ns, 20 pulse) increased avermectin (anthelmintic and insecticidal agent) production in *Streptomyces avermitilis* by 42% and reduced the time needed for reaching a plateau in the fermentation process from 5 to 7 days [60]. For other related studies see [61].

- **Food industry**: Microalgae are a novel food ingredient of increasing interest as they can be grown on non-arable lands and fixates $CO_2$ when grown photoautotrophically. Treatment with nsPEF (5–100 kV/cm, 2–100 ns) reduced total bacterial contamination $>\log_{10}$ in *Chlorella vulgaris* cultures without compromising the microalgae. For related studies see [62][63].

- **Seed germination**: nsPEF (10–30 kV/cm, 100 ns, 20 pulses) application significantly affected seed germination and pre-growth of *Haloxylon ammodendron*. This is probably due to the exogenous and endogenous NO generated in the nsPEF seed-treatment system [64]. For related studies see [65][66].

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