The Electrode Modality Development in Pulsed Electric Field Treatment Facilitates Biocellular Mechanism Study and Improves Cancer Ablation Efficacy

Chao Cen\(^1\) and Xinhua Chen\(^{1,2}\)

\(^1\)The Key Laboratory of Combined Multi-Organ Transplantation, Ministry of Public Health and The Department of Hepatobiliary and Pancreatic Surgery, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China
\(^2\)The Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China

Correspondence should be addressed to Xinhua Chen; xinhua.chen@zju.edu.cn

Received 25 January 2017; Accepted 15 March 2017; Published 7 May 2017

Academic Editor: Feng-Huei Lin

Copyright © 2017 Chao Cen and Xinhua Chen. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Pulsed electric field treatment is now widely used in diverse biological and medical applications: gene delivery, electrochemotherapy, and cancer therapy. This minimally invasive technique has several advantages over traditional ablation techniques, such as nonthermal elimination and blood vessel spare effect. Different electrodes are subsequently developed for a specific treatment purpose. Here, we provide a systematic review of electrode modality development in pulsed electric field treatment. For electrodes invented for experiment in vitro, sheet electrode and electrode cuvette, electrodes with high-speed fluorescence imaging system, electrodes with patch-clamp, and electrodes with confocal laser scanning microscopy are introduced. For electrodes invented for experiment in vivo, monopolar electrodes, five-needle array electrodes, single-needle bipolar electrode, parallel plate electrodes, and suction electrode are introduced. The pulsed electric field provides a promising treatment for cancer.

1. Introduction

In recent years, pulsed electric field treatment has been gaining extensive attention in virtue of its biological and medical applications, such as gene delivery [1–5], electrochemotherapy [6–11], and cancer therapy [12–18]. One advantage of pulsed electric field treatment, making it distinctive from other physical techniques, is the ability to destroy tissues or tumors in a nonthermal manner [19, 20]. Consequently, pulsed electric field treatment makes it possible to preserve sensitive tissues intact, such as blood vessels and axons [21, 22]. Furthermore, this minimally invasive technique allows the possibility of regeneration with healthy cells and tissues in the treatment region and leaves a minimal scar [23]. With the aid of ultrasound, CT, or MRI, pulsed electric field treatment could be monitored in real time, which helps improve the treatment efficiency immensely [24–26].

Conventional appliance consists of three parts: pulse generator, electrodes, and connection links between them. The pulse generator produces square wave pulses at regular intervals. Amplitude, pulse width, period, and phase delay are the primary parameters to determine the shape of the output waveform. Electric field strength, depending on the amplitude of the pulse and the distance between the electrodes, is often crucial for completed treatment effect [27]. When electrodes are unsuitable, the strength in a certain target area is insufficient, resulting in
incomplete treatment effects. As the literature focusing on this research field is scarce, an overview of electrodes appears very timely. This review can be used to improve electrodes for individual-based treatment and guide new electrode designs.

We incorporate papers that are representative of existing technology and indicative of future directions, based on the massive amount of literature about electrode assemblies used in pulsed electric field treatment. As current medical applications of pulsed electric fields are mainly concentrated on cell and tissue treatment, this review is divided into two sections: section A, electrodes invented for in vitro (cell response to the treatment), and section B, electrodes invented for in vivo (tissue response to the treatment).

2. Part I: Electrode Invented for Experiment In Vitro

2.1. Traditional Electrodes for Membrane Charging Measurement. A cell is often described as a conductive body (the cytoplasm) surrounded by a dielectric layer (the surface membrane). When a cell is applied to an electric field in a conductive medium, electric charges accumulate at the cell membrane and consequently form a voltage across the membrane. Frey et al. [28] designed a system (Figure 1) to investigate plasma membrane voltage changes in response to nanosecond pulsed electric fields. Jurkat cells were stained with Annine-6 (a novel voltage-sensitive hemicyanine dye with a subnanosecond temporal response, optically measuring the changes in transmembrane voltage of excitable cells [29]) and then exposed to an electric field of 95 kV/cm for 60 ns. Then, the illumination was provided by a dye laser whose wavelength was close to the excitation maximum of Annine-6 and pulse duration was far less than the duration of nanosecond pulsed electric field exposure. Taking the advantage of the technical progress, membrane voltage changes with time were monitored. The results showed a strong asymmetry between the anodic and cathodic poles: the membrane facing the anodic pole reached values of 1.6 V after 15 ns, compared with only 0.6 V at the cathodic pole at the same time (figure not shown). These facilities make it possible to monitor the real-time cell membrane voltage changes in site so that the firsthand direct cell response can be recorded objectively.

Another nonignorable conclusion drawn from this electrode model is intracellular effect of nanosecond pulsed electric fields—almost all the voltage was applied across the interior of the cell. This phenomenon has been confirmed by several studies [30–34]. Among all the complex cascade of events, including chromatin condensation and nucleosomal DNA fragmentation, disruption of mitochondrial membrane potential is often considered to be involved in activation of caspase-mediated apoptosis. Thus, with this electrode model, the mitochondrial membrane potential can be also detected with fluorescent dye rhodamine 123 [35], TMRE [36], DiOC₆ [3], JC-1, and TMRM.

When plasma membrane voltage exceeds a critical value (about 500 mV), transmembrane pores form, which is known as electroporation [37]. It is found that the pore size is a function of the duration of the pulse. Microsecond pulses generate large pores (conventional electroporation), which are big enough to deliver macromolecules, such as DNA [1–5], dyes [38], and drugs [39]. In contrast, the pores generated by a nanosecond pulsed electric field are only about 1 nanometer wide, which are often named as “nanopores.” Nanopores have little permeability to propidium iodide (a macromolecule dye used to stain DNA) [40] but show permeability to small inorganic ions, distinguishing from conventional electroporation. This hypothesis was supported by a series of researches, cellular uptake of Tl³⁺ [41], Cu²⁺ [42], and Ca²⁺ [43, 44], among which cellular uptake of Ca²⁺ is mostly related to biological functions. Increased intracellular Ca²⁺ may be involved in triggering mitochondrial apoptosis pathway via cytochrome c [45–48].

2.2. Sheet Electrode and Electrode Cuvette. Several previous studies have been designed to figure out whether Ca²⁺ comes from intracellular Ca²⁺ pools or extracellular solutions. Verrier et al. [49] demonstrated in 2003 that nsPEF triggers Ca²⁺ release from the endoplasmic reticulum in Jurkat cells, and White et al. [40] confirmed it in HL-60 cells in 2004. However, Craviso et al. [50] did not find Ca²⁺ release from the endoplasmic reticulum using adrenal chromaffin cells. They fabricated microelectrode chambers on a glass microscope slide with gold electrodes (Figure 2). With the indication of the calcium-sensitive fluorescence indicator Calcium Green, the intracellular calcium level of adrenal chromaffin cells could be detected. Consistent with previous findings, the rise of intracellular calcium depends on extracellular calcium. In parallel experiments, chromaffin cells were transferred to electroporation cuvettes (Figure 3) for nanosecond pulsed electric field exposure. The release of norepinephrine and epinephrine was determined by high-performance liquid chromatography coupled with electrochemical detection. It was concluded that nanosecond pulsed electric field could elicit calcium-dependent catecholamine release in chromaffin cells.

Sheet electrodes are characterized by two parallel pieces of gold foil (5 mm wide; 25 μm thick; 100 μm apart), placed partly overlapped. This design ensures a homogeneous electric field and maximally eliminates “fringing effects” due to the sharp edges of the microchamber electrodes. When a
large number of cells need to be treated, the electroporation cuvette (1 mm electrode) may be the first choice. It could contain nearly 10^6 cells in a 200 μl suspension and provide a homogeneous electric field, but “fringing effects” seems hard to avoid.

2.3. The Cylinder Tungsten Electrodes with High-Speed Fluorescence Imaging System. As stated above, Craviso et al. put forward that intracellular Ca^{2+} primarily came from extracellular pools, but the detection precision was limited to 1 second. To find out what happens in the first 1 second, Beier et al. [43] set up a high-speed fluorescence imaging system (Figure 4) to monitor Ca^{2+} movement and proposed the opposite opinion: intracellular calcium pools seem much important. Rodent neuroblastoma cells were cultured on a glass-bottom poly-L-lysine coated dish and incubated in exposure buffer for 30 min prior to nanosecond pulsed electric field, avoiding interference of changes of buffer solution. An argon-krypton ion laser tuned to 488 nm was employed to excite the intracellular fluorescent dye. Different conventional imaging methods, such as epifluorescence and confocal microscopy [51], are too slow to capture the possibly instant Ca^{2+} influx. Timing of the imaging system, laser irradiation, and nanosecond pulsed electric field delivery was controlled by using a digital delay generator (Stanford Research Systems), making it feasible to record Ca^{2+} influx in milliseconds. The results showed that an increased Ca^{2+} concentration was visible after 3.5 ms. In addition, it provides evidence that intracellular Ca^{2+} concentration increases in the absence of extracellular calcium; alluding intracellular calcium pools play a major role in intracellular Ca^{2+} concentration increase when exposed to nanosecond pulsed electric field. These results benefit mostly from the utilization of high-speed fluorescence imaging system and visualize the Ca^{2+} in milliseconds. Taking advantage of this appliance, other micromolecules can be detected in the very early stage such as Tl^{3+} and Cu^{2+}.

2.4. Tungsten Electrodes in Combination with Patch-Clamp. Another important question is how long the nanopores last. Patch-clamp was employed to explore nanopores on cell membrane and provide new insights [52]. Cells were
suspended on a cover slip and then tungsten electrodes were positioned with a micromanipulator at the sides of the cell (Figure 5). To avoid the interference of patch-clamp to the cell exposed to nanosecond pulsed electric field, patch pipette keeps intact with the cell until 50 s (80–120 s on average) after the exposure. Even with such a long delay, profound decrease of cell membrane resistance ($R_m$) was still detected, accompanied by the loss of the membrane potential. The early studies estimated that pulsed electric fields cause cell membrane permeabilization opening big pores in cell membrane, but currently, patch clamp technology revealed that ultrashort intensive pulsed electric field opens small plasma membrane pores [33, 49, 53]. Similar to a conventional electrodes design, tungsten electrodes generated homogeneous electric fields. This new finding mainly benefits from the application of patch-clamp, which could detect electric current through the nanopores in a much smaller order of magnitude. Patch-clamp provides a precise approach to measure single or multiple ion channel responses to nanosecond pulsed electric field treatment. Some works carrying on demonstrate that nanosecond pulsed electric field may have the effect of not only long-term permeabilization but also inhibition of voltage-gated $I_{Na}$ and $I_{Ca}$ [54, 55].

2.5. Electrodes Assembled with Confocal Laser Scanning Microscopy. As mentioned in Section 2.1, nanosecond pulsed electric field can penetrate into the cell interior, disturbing mitochondrial membrane potential. However, experiments visualizing the internal membrane to monitor the real-time response to the nanosecond pulsed electric field are rare. Confocal laser scanning microscopy is often deemed to be competent to view subcellular structures. Berghöfer et al. then designed confocal microscopy to the nanosecond pulsed electric field system [51]. Tobacco wild cell line (BY-2) was used that expressed GFP in fusion with markers for tubulin, endoplasmic reticulum, and actin filaments. As seen in Figure 6, confocal laser scanning microscopy was assembled to conventional nanosecond pulsed electric field treatment system, and interior structures in selected depths were imaged in high-resolution. Microtubules disorder, actin disassembly, and nuclear envelope disintegration were observed; insinuating nanosecond pulsed electric field affects not only the internal membrane but also cytoskeleton structures. Confocal microscopy is featured for its ability to acquire in-focus images from selected depths. Images are acquired point by point and reconstructed with a computer, allowing three-dimensional reconstructions of the cell. So, this invention provides a method to view subcellular structures responses to nanosecond pulsed electric field in three-dimension format.

3. Part II: Electrode Invented for Experiment In Vivo

Beside resection or transplantation surgery, the focal ablation techniques such as cryosurgery, microwave (MW), laser interstitial thermal therapy (LITT), high-intensity focused ultrasound (HIFU), and radiofrequency ablation (RFA) remain to be fundamental means for cancer treatment. Cryosurgery freezes the tissue as low as −40°C to cause tissue necrosis. MW, LITT, HIFU, and RFA often heat the tissue to be more than 60°C, producing cell death and coagulative necrosis [56]. However, these thermal techniques cause inevitable complications, such as incomplete treatment of vascularized tissue (“sink effect” of the blood), possible damage to the blood vessel [57], production of unexpected scar [58], and exclusion of tumor lying within 1 cm of the overlying skin for fear of burning of the skin or muscle [59]. As a non-thermal technique, nanosecond pulsed electric field ablation has a better performance in avoiding these thermal complications, which raises the tissue temperature by only 0.3°C [60].

3.1. Traditional Monopolar Electrodes. The prevailing electrodes used for the treatment of solid cancer consist of two needles, one is charged and the other is grounded (as shown in Figure 7). The electrodes were chosen to be 1 mm in diameter and separated 0.8 cm apart [61]. The advantage of monopolar electrodes is the flexibility for electrode layout to optimize treatment of a targeted region, satisfying the requirements of individual-based treatment. In addition,
Figure 6: Electrodes assembled with confocal laser scanning microscopy (cited from [51], Figure 1).
monopolar electrodes are often used as a standard model to measure the varying strength around the needle in space.

3.2. Five-Needle Array Electrodes. To enlarge the treatment area, five-needle array was applied [62]. The needle array (Figure 8) was made using 30 gauge hypodermic needles (300 μm diameter) extending 2 mm from a Teflon base. The center needle forms the anode; the other four surrounding needles spaced 4 mm from the center electrode form the cathode. To avoid flashover between needles and accompanying skin damage, skin was coated with vegetable oil. Malignomas shrinks to about 10% of original size after the first treatment and complete remission is observed after the second treatment. The effect of 5-needle array was also confirmed in another study [63] in which bioelectrical field ablation efficiently eliminated papillomas and squamous cell carcinoma in vivo from skin of mice (figure not shown). However, the tumor treated has to be fit for the size of five-needle array electrodes, which hinders the popularization in other areas.

3.3. Single-Needle Bipolar Electrode. Although the larger electrode with multiple needles could cause enlarged electric field coverage, problems such as placement complexity and more physical damage from electrode penetration come up consequently. So, a single needle bipolar electrode was used [64]. The electrode is divided into two parts: an electrode body coated with an insulating layer and a tip containing two electrically conductive surfaces separated by an additional insulating layer (Figure 9). To detect the effects of this single needle bipolar electrode, bioluminescent images and histological examination were performed. The results showed that the treatment achieved breast cancer regression in mice. Featured for its convenience, single-needle bipolar electrode generates an inhomogenous electric field, which means electric field may not cover the tumor completely. Operators have to apply more pulse times to ensure the complete elimination. Taking advantage of its convenience, if this single needle bipolar electrode could be further improved, it will be a promising alternative of bioelectrical field ablation.

3.4. The Noninvasive Parallel Plate Electrodes. As techniques improve, noninvasive surgery and instruments are in great

Figure 7: The monopolar electrodes (cited from [61], Figure 1).

Figure 8: Needle array electrode and electric field pattern (cited from [62], Figure 2).

Figure 9: The single-needle bipolar electrode (cited from [64], Figure 1).
need. The noninvasive parallel plate electrodes were designed by Nuccitelli et al. [62]. The parallel plate electrodes (Figure 10) were made from stainless steel, and the size could be adjusted according to the treated tumor from 3 mm to 5 mm in diameter. Each tumor was placed between two plates with a separation of 0.5–1 mm. A thick layer of conductive agar coated the electrodes to separate skin from the electrodes. Tumor shrank in a large extent in spite of no elimination. A black scab appeared on the stratum corneum as the skin was exposed to the electric pulse fields. The main advantage of parallel plate electrodes is that they could deliver a more equally distributed electric field and ensure the target area is exposed to the similar electric field strength.

3.5. Suction Electrode. Similar to parallel plate electrodes, Nuccitelli et al. designed suction electrodes (Figure 11) for noninvasive treatment [60, 65]. Suction electrodes are plastic cylinders with a cup-shaped opening at one end with an inner diameter of 4 mm and a depth of 2 mm. At the base of each cup were small holes, allowing a suction to be applied to pull the tumor into the cavity. The treatment area was highly localized to the tissues in the suction electrodes. The suction removes air from the cavity, then gases are not available for ionization by the high electric fields applied that could lead to arcing. Similar to five-needle array electrodes, the limitation was that treatment size was subjected to the “electrode size” and the treatment area was limited to the subcutaneous tissue.

3.6. Electrodes Invented for Brain Treatment. When applied to clinical application, pulsed electric field was initially used to destroy substantial volumes of tissue [63, 65–68], then it extended the application to liver [24, 60, 69], prostate [21], and sarcoma tumors [19]. Garcia et al. [27] provided a preliminary study of brain cancer therapy with conventional monopolar electrodes (Figure 12). The probes were 1 mm in diameter with an insulating sheath, where 0.5-cm-long tips were exposed in contact with the tissue. Combining with previous [25] and latter [70] relevant researches by this group,
the results showed the safety of bioelectrical field ablation in the brain and the affected lesion volume was correlated with the applied voltage. Intracranial approach in neurosurgery is still in the initial stage, and this new invention has shown advantages superior to the invasive resection: (1) the small electrode size ensures the procedure minimally invasive and adaptable to almost any neuroanatomical location under the guidance of ultrasonography and MR imaging; (2) bioelectrical field ablation makes it feasible to create a sharply delineated volume of ablated tissue with submillimeter resolution in the brain, where tumors are often deep-seated and well-circumscribed; and (3) blood vessel spare effect maximally protects the sensitive tissues adjacent to the tumor.

4. Conclusion

Here, we provide a brief review of the electrode reported in the pulsed electric field treatments in vitro and in vivo. The specific applications, the advantages, and the disadvantages are also discussed. It was reported [71] that a first-in-human trial nanoelectroablation therapy for basal cell carcinoma has been recently carried out. The results confirmed the safety and feasibility of the pulsed electric field ablation. The electrodes are expected to attain the maximum efficiency and minimal complications.

Abbreviations

CT: Computed tomography
DiOC<sub>6</sub>: 3,3′-Dihexyloxycarbocyanine iodide
DNA: Deoxyribonucleic acid
GFP: Green fluorescent protein
HIFU: High-intensity focused ultrasound
JC-1: 5,5′,6,6′-Tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide
LITT: Laser interstitial thermal therapy
MRI: Magnetic resonance imaging
MW: Microwave
RFA: Radiofrequency ablation
R<sub>m</sub>: Cell membrane resistance
TMRM: Tetramethylrhodamine.

Conflicts of Interest

All authors certify that this manuscript has not been published in whole or in part nor is it being considered for publication elsewhere. The authors have no conflicts of interest to declare.

Authors’ Contributions

Chao Cen reviewed the published manuscripts and Xinhua Chen summarized the development.

Acknowledgments

The research is supported by the National Natural Science Foundation of China (no. 81372425) and the Key Lab Project of the Xinjiang Science and Technology Bureau (2014KL002).

References

[1] B. Ferraro, Y. L. Cruz, D. Coppola, and R. Heller, "Intradermal delivery of plasmid VEGF(165) by electroporation promotes wound healing," Molecular Therapy, vol. 17, no. 4, pp. 651–657, 2009.

[2] L. Heller, M. J. Jaroszeski, D. Coppola, C. Pottinger, R. Gilbert, and R. Heller, "Electrically mediated plasmid DNA delivery to hepatocellular carcinomas in vivo," Gene Therapy, vol. 7, no. 10, pp. 826–829, 2000.

[3] A. M. Bodles-Brakhop, R. Heller, and R. Draghia-Akli, "Electroporation for the delivery of DNA-based vaccines and immunotherapeutics: current clinical developments," Molecular Therapy, vol. 17, no. 4, pp. 585–592, 2009.

[4] B. D. Livingston, S. F. Little, A. Luxemburg, B. Ellefsen, and D. Hannaman, "Comparative performance of a licensed anthrax vaccine versus electroporation based delivery of a PA encoding DNA vaccine in rhesus macaques," Vaccine, vol. 28, no. 4, pp. 1056–1061, 2010.

[5] A. Donate, D. Coppola, Y. Cruz, and R. Heller, "Evaluation of a novel non-penetrating electrode for use in DNA vaccination," PLoS One, vol. 6, no. 4, article e19181, 2011.

[6] M. Okino and H. Mohri, "Effects of a high-voltage electrical impulse and an anticancer drug on in vivo growing tumors," Japanese Journal of Cancer Research, vol. 78, no. 12, pp. 1319–1321, 1987.

[7] S. Orlowski, J. Belehradek Jr., C. Paoletti, and L. M. Mir, "Transient electroporation of cells in culture. Increase of the cytotoxicity of anticancer drugs," Biochemical Pharmacology, vol. 37, no. 24, pp. 4727–4733, 1988.

[8] R. Heller, M. J. Jaroszeski, L. F. Glass et al., "Phase I/II trial for the treatment of cutaneous and subcutaneous tumors using electrochemotherapy," Cancer, vol. 77, no. 5, pp. 964–971, 1996.

[9] J. Gehl, T. Skovsgaard, and L. M. Mir, "Enhancement of cytotoxicity by electroporation: an improved method for screening drugs," Anti-Cancer Drugs, vol. 9, no. 4, pp. 319–325, 1998.

[10] R. Heller, D. Coppola, C. Pottinger, R. Gilbert, and M. J. Jaroszeski, "Effect of electrochemotherapy on muscle and skin," Technology in Cancer Research & Treatment, vol. 1, no. 5, pp. 385–392, 2002.

[11] A. Gothelf, L. M. Mir, and J. Gehl, "Electrochemotherapy: results of cancer treatment using enhanced delivery of bleomycin by electroporation," Cancer Treatment Reviews, vol. 29, no. 5, pp. 371–387, 2003.

[12] V. Munoz Madero and P. G. Ortega, "Electrochemotherapy for treatment of skin and soft tissue tumours. Update and definition of its role in multimodal therapy," Clinical & Translational Oncology, vol. 13, no. 1, pp. 18–24, 2011.

[13] A. Testori, G. Tosti, C. Martinoli et al., "Electrochemotherapy for cutaneous and subcutaneous tumor lesions: a novel therapeutic approach," Dermatologic Therapy, vol. 23, no. 6, pp. 651–661, 2010.

[14] X. J. Yang, J. Li, C. X. Sun, F. Y. Zheng, and L. N. Hu, "The effect of high frequency steep pulsed electric fields on in vitro and in vivo antitumor efficiency of ovarian cancer cell line skov3 and potential use in electroporation," Journal of Experimental & Clinical Cancer Research, vol. 28, no. 1, p. 53, 2009.
[15] E. D. Kirson, V. Dbaly, F. Tovarys et al., “Alternating electric fields arrest cell proliferation in animal tumor models and human brain tumors,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 24, pp. 10152–10157, 2007.

[16] B. R. Persson, C. Baureus Koch, G. Graffstrom, P. E. Engstrom, and L. G. Salford, “A model for evaluating therapeutic response of combined cancer treatment modalities: applied to treatment of subcutaneously implanted brain tumors (N32 and N29) in Fischer rats with pulsed electric fields (PEF) and 60Co-gamma radiation (RT),” *Technology in Cancer Research & Treatment*, vol. 2, no. 5, pp. 459–470, 2003.

[17] Y. Kubota, T. Nakada, and I. Sasagawa, “Treatment of rat bladder cancer with electrochemotherapy in vivo,” *Methods in Molecular Medicine*, vol. 37, pp. 293–298, 2000.

[18] G. A. Hofmann, S. B. Dev, S. Dimmer, and G. S. Nanda, “Electroporation therapy: a new approach for the treatment of head and neck cancer,” *IEEE Transactions on bio-Medical Engineering*, vol. 46, no. 6, pp. 752–759, 1999.

[19] B. Al-Sakere, F. Andre, C. Bernat et al., “Tumor ablation with irreversible electroporation,” *PloS One*, vol. 2, no. 11, article e1135, 2007.

[20] R. V. Davalos, I. L. Mir, and B. Rubinsky, “Tissue ablation with irreversible electroporation,” *Annals of Biomedical Engineering*, vol. 33, no. 2, pp. 223–231, 2005.

[21] G. Onik, P. Mikus, and B. Rubinsky, “Irreversible electroporation: implications for prostate ablation,” *Technology in Cancer Research & Treatment*, vol. 6, no. 4, pp. 295–300, 2007.

[22] B. Rubinsky, “Irreversible electroporation in medicine,” *Technology in Cancer Research & Treatment*, vol. 6, no. 4, pp. 255–260, 2007.

[23] B. Rubinsky, G. Onik, and P. Mikus, “Irreversible electroporation: a new ablation modality—clinical implications,” *Technology in Cancer Research & Treatment*, vol. 6, no. 1, pp. 37–48, 2007.

[24] E. W. Lee, C. T. Loh, and S. T. Kee, “Imaging guided percutaneous irreversible electroporation: ultrasound and immunohistological correlation,” *Technology in Cancer Research & Treatment*, vol. 6, no. 4, pp. 287–294, 2007.

[25] P. A. Garcia, J. H. Rossmeiscl Jr., I. Robertson, T. L. Ellis, and R. V. Davalos, “Pilot study of irreversible electroporation for intracranial surgery,” *Conference Proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, vol. 2009, pp. 5819–5822, 2007.

[26] W. Ren and S. J. Beebe, “An apoptosis targeted stimulus with nanosecond pulsed electric fields (nsPEFs) in E4 squamous cell carcinoma,” *Apatosis*, vol. 16, no. 4, pp. 382–393, 2011.

[27] J. Weaver, *The Biomedical Engineering Handbook*, pp. 1431–1440, CRC Press and IEEE Press, Boca Raton, Florida, USA, 1995.

[28] K. Hashimoto, N. Tatsumi, and K. Okuda, “Introduction of phalloidin labeled with fluorescein isothiocyanate into living polymorphonuclear leukocytes by electroporation,” *Journal of Biochemical and Biophysical Methods*, vol. 19, no. 2–3, pp. 143–153, 1989.

[29] M. Kambe, D. Arita, H. Kikuchi et al., “Enhancing the effect of anticancer drugs against the colorectal cancer cell line with electroporation,” *The Tohoku Journal of Experimental Medicine*, vol. 180, no. 2, pp. 161–171, 1996.

[30] J. A. White, P. F. Blackmore, K. H. Schoenbach, and S. J. Beebe, “Stimulation of capacitative calcium entry in HL-60 cells by nanosecond pulsed electric fields,” *The Journal of Biological Chemistry*, vol. 279, no. 22, pp. 22964–22972, 2004.

[31] A. G. Pakhomov, A. M. Bowman, B. L. Ibyey, F. M. Andre, O. N. Pakhomova, and K. H. Schoenbach, “Lipid nanopores can form a stable, ion channel-like conduction pathway in cell membrane,” *Biochemical and Biophysical Research Communications*, vol. 385, no. 2, pp. 181–186, 2009.

[32] G. H. Wang, L. Wang, Y. J. Han, S. Zhou, and X. Y. Guan, “Nanopore detection of copper ions using a polystyrene probe,” *Biosensors & Bioelectronics*, vol. 53, pp. 453–458, 2014.

[33] H. T. Beier, C. C. Roth, G. P. Tolstykh, and B. L. Ibyey, “Resolving the spatial kinetics of electric pulse-induced ion release,” *Biochemical and Biophysical Research Communications*, vol. 423, no. 4, pp. 863–866, 2012.

[34] S. S. Scarlett, J. A. White, P. F. Blackmore, K. H. Schoenbach, and J. F. Kolb, “Regulation of intracellular calcium concentration by nanosecond pulsed electric fields,” *Biochimica et Biophysica Acta-Abiomembranes*, vol. 1788, no. 5, pp. 1168–1175, 2009.
[45] S. J. Beebe, P. M. Fox, L. J. Rec, E. L. Willis, and K. H. Schoenbach, “Nanosecond, high-intensity pulsed electric fields induce apoptosis in human cells,” FASEB Journal, vol. 17, no. 11, pp. 1493–1495, 2003.

[46] Y. P. Ow, D. R. Green, Z. Hao, and T. W. Mak, “Cytochrome c: functions beyond respiration,” Nature Reviews. Molecular Cell Biology, vol. 9, no. 7, pp. 532–542, 2008.

[47] G. Kroemer, L. Galluzzi, and C. Brenner, “Mitochondrial membrane permeabilization in cell death,” Physiological Reviews, vol. 87, no. 1, pp. 99–163, 2007.

[48] W. Ren, N. M. Sain, and S. J. Beebe, “Nanosecond pulsed electric fields (nsPEFs) activate intrinsic caspase-dependent and caspase-independent cell death in Jurkat cells,” Biochemical and Biophysical Research Communications, vol. 421, no. 4, pp. 808–812, 2012.

[49] P. T. Vernier, Y. Sun, L. Marcu, S. Salemi, C. M. Craft, and M. A. Gundersen, “Calcium bursts induced by nanosecond electric pulses,” Biochemical and Biophysical Research Communications, vol. 310, no. 2, pp. 286–295, 2003.

[50] G. L. Craviso, P. Chatterjee, G. Maalouf et al., “Nanosecond electric pulse-induced increase in intracellular calcium in adrenal chromaffin cells triggers calcium-dependent catecholamine release,” IEEE Transactions on Dielectrics and Electrical Insulation, vol. 16, no. 5, pp. 1294–1301, 2009.

[51] T. Berghöfer, C. Eing, B. Flickinger et al., “Nanosecond electric pulses trigger actin responses in plant cells,” Biochemical and Biophysical Research Communications, vol. 387, no. 3, pp. 590–595, 2009.

[52] A. G. Pakhomov, J. F. Kolb, J. A. White, R. P. Joshi, S. Xiao, and K. H. Schoenbach, “Long-lasting plasma membrane permeabilization in mammalian cells by nanosecond pulsed electric field (nsPEF),” Bioelectromagnetics, vol. 28, no. 8, pp. 655–663, 2007.

[53] P. T. Vernier, Y. Sun, L. Marcu, C. M. Craft, and M. A. Gundersen, “Nanoelectropulse-induced phosphatidylserine translocation,” Biophysical Journal, vol. 86, no. 6, pp. 4040–4048, 2004.

[54] V. Nesin, A. M. Bowman, S. Xiao, and A. G. Pakhomov, “Cell permeabilization and inhibition of voltage-gated Ca2+ and Na+ channel currents by nanosecond pulsed electric field,” Bioelectromagnetics, vol. 33, no. 5, pp. 394–404, 2012.

[55] A. O. Verkerk, A. C. van Ginneken, and R. Wilders, “Sodium current inhibition by nanosecond pulsed electric field (nsPEF)—fact or artifact?” Bioelectromagnetics, vol. 34, no. 2, pp. 162–164, 2013.

[56] E. M. Knavel and C. L. Brace, “Tumor ablation: common modalities and general practices,” Techniques in Vascular and Interventional Radiology, vol. 16, no. 4, pp. 192–200, 2013.

[57] J. Kai, S. Ming, L. Yang et al., “Complete radio frequency ablation of hepatocellular carcinoma adjacent to the main bile duct and blood vessels between the first and the second hepatic portal,” Cell Biochemistry and Biophysics, vol. 66, no. 2, pp. 397–402, 2013.

[58] N. K. Janzen, K. T. Perry, K. R. Han et al., “The effects of intentional cryoablation and radio frequency ablation of renal tissue involving the collecting system in a porcine model,” The Journal of Urology, vol. 173, no. 4, pp. 1368–1374, 2005.

[59] S. E. Singleton, B. D. Fornage, N. Sniege et al., “Radiofrequency ablation of early-stage invasive breast tumors: an overview,” Cancer Journal, vol. 8, no. 2, pp. 177–180, 2002.

[60] R. Nuccitelli, K. Tran, K. Lui et al., “Non-thermal nanoelectroablation of UV-induced murine melanomas stimulates an immune response,” Pigment Cell & Melanoma Research, vol. 25, no. 5, pp. 618–629, 2012.

[61] R. E. Neal 2nd and R. V. Davalos, “The feasibility of irreversible electroporation for the treatment of breast cancer and other heterogeneous systems,” Annals of Biomedical Engineering, vol. 37, no. 12, pp. 2615–2625, 2009.

[62] R. Nuccitelli, U. Pliquett, X. Chen et al., “Nanosecond pulsed electric fields cause melanomas to self-destruct,” Biochemical and Biophysical Research Communications, vol. 343, no. 2, pp. 351–360, 2006.

[63] D. Yin, W. G. Yang, J. Weissberg et al., “Cutaneous papilloma and squamous cell carcinoma therapy utilizing nanosecond pulsed electric fields (nsPEF),” PLoS One, vol. 7, no. 8, article e43891, 2012.

[64] R. E. Neal 2nd, R. Singh, H. C. Hatcher, N. D. Kock, S. V. Torti, and R. V. Davalos, “Treatment of breast cancer through the application of irreversible electroporation using a novel minimally invasive single needle electrode,” Breast Cancer Research and Treatment, vol. 123, no. 1, pp. 295–301, 2010.

[65] R. Nuccitelli, K. Tran, S. Sheikh, B. Athos, M. Kreis, and P. Nuccitelli, “Optimized nanosecond pulsed electric field therapy can cause murine malignant melanomas to self-destruct with a single treatment,” International Journal of Cancer, vol. 127, no. 7, pp. 1727–1736, 2010.

[66] X. Chen, R. James Swanson, J. F. Kolb, R. Nuccitelli, and K. H. Schoenbach, “Histopathology of normal skin and melanomas after nanosecond pulsed electric field treatment,” Melanoma Research, vol. 19, no. 6, pp. 361–371, 2009.

[67] X. Chen, R. J. Swanson, K. H. Schoenbach, S. Yin, and S. Zheng. “Histopathological follow-up by tissue micro-array in a survival study after melanoma treated by nanosecond pulsed electric fields (nsPEF),” The Journal of Dermatological Treatment, vol. 22, no. 3, pp. 153–161, 2011.

[68] X. Chen, K. H. Schoenbach, S. Zheng, and R. J. Swanson, “Comparative study of long- and short-pulsed electric fields for treating melanoma in an in vivo mouse model,” In Vivo, vol. 25, no. 1, pp. 23–27, 2011.

[69] J. F. Edd, L. Horowitz, R. V. Davalos, L. M. Mir, and R. Rubinsky, “In vivo results of a new focal tissue ablation technique: irreversible electroporation,” IEEE Transactions onbio-Medical Engineering, vol. 53, no. 7, pp. 1409–1415, 2006.

[70] T. L. Ellis, P. A. Garcia, J. H. Rossmeisl Jr., N. Henao-Guerrero, J. Robertson, and R. V. Davalos, “Nonthermal irreversible electroporation for intracranial surgical applications. Laboratory investigation,” Journal of Neurosurgery, vol. 114, no. 3, pp. 681–688, 2011.

[71] R. Nuccitelli, R. Wood, M. Kreis et al., “First-in-human trial of nanoelectroablation therapy for basal cell carcinoma: proof of method,” Experimental Dermatology, vol. 23, no. 2, pp. 135–137, 2014.