Nonthermal Irreversible Electroporation: Fundamentals, Applications, and Challenges

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***Abstract*—Tissue ablation is an essential procedure for the treatment of many diseases. In the last decade, a nonthermal tissue ablation using intensive pulsed electric fields, called nonthermal irreversible electroporation (NTIRE), has rapidly emerged. The exact mechanisms responsible for cell death by NTIRE, however, are currently unknown. Nevertheless, the technique's remarkable ability to ablate tissue in the proximity of larger blood vessels, to preserve tissue architecture, short procedure duration, and shortened postoperative recovery period rapidly moved NTIRE from bench to bed side. This work provides an overview on the development of NTIRE, its current state-of-the-art, challenges, and future needs.**

***Index Terms*—Irreversible electroporation, minimally invasive surgery, nonthermal ablation, pulsed electric fields.**

I. Introduction

TISSUE ablation is an essential surgical method for disease treatment and anatomical correction. Classic methods to destroy tissues in the context of medical therapy have included: radio frequency, cryo, microwave, ultrasonic, laser, and chemical ablation [1]. The volumes of tissue ablated by these methods, however, are limited by blood perfusion, especially in proximity to large blood vessels [2]. Intense blood flow leads to thermal sinks, which limit the thermal ablation methods; the flow also leads to washing-out, or clearance, which limits the chemical ablation.

In the recent decade, an alternative, nonthermal-pulsed-electric field method, coined nonthermal irreversible electroporation (NTIRE), has emerged. In NTIRE, externally applied pulsed electric fields cause irreversible damage to cells by affecting the cell membrane, sparing, however, tissue scaffold, large blood vessels and other tissue structures [3]–[5]. Unlike other ablation methods, NTIRE is not affected by the local blood flow, and therefore it kills cells also in the margin of the large blood vessels [6]. Furthermore, reduced posttreatment inflammation [7] promotes reduced scarring, observed for several tissues [5], [8]. In this work, we review the development, state-of-the-art and challenges ahead of NTIRE in the context of medical application.

A. Brief History of NTIRE in Medicine

The effects of pulsed electric fields on biological matter date back over 250 years. In the middle of the 18th century Nollet reported the first systematic observations of the appearances of red spots on animal and human skin exposed to the electric sparks [9]. The electrical conductivity changes in nerves, damaged by electric fields, as reported in [10]–[12], can probably be explained in the context of NTIRE. Moreover, NTIRE might be the explanation of nonthermal-pulsed-electric-field sterilization, as first reported by Fuller in 1898 [13], reviewed in [14]. In addition, NTIRE probably explains "electroplasmolyses," a plant tissue lyses process, developed by Flaumenbaum and Zagorulko, in the USSR and Doevenspeck in Germany [15]–[17], reviewed in [18]. The seminal work of Neumann and colleagues in [19] led to important applications of reversible electroporation for cell electrotransfection in laboratories and two clinical applications: electrochemotherapy and electrogenetherapy, recently reviewed in [19] and [20].

In addition to studies related to basic science, critical discoveries were also made in clinics. Lee and colleagues, suggested that the tissue electric trauma has a nonthermal, irreversible electroporation nature of damage, in addition to thermal effects [22], reviewed in [23].

These basic and clinical discoveries served as a foundation to the first theoretical paper on NTIRE published by Davalos Mir and Rubinsky in [24]. Here, the authors proposed that pulsed electric fields can nonthermally ablate clinically relevant volumes of tissue [24]. The experimental and clinical results immediately followed and showed that pulsed electric fields can indeed nonthermally ablate tissues. Moreover, the procedure preserved major components of tissue architecture which suggest a possibility for the healthy tissue regeneration with reduced scar formation [25].

B. Fundamental Principles of Irreversible Electroporation

Externally applied electric fields increase cell membrane permeabilization, a phenomenon coined "electroporation" [19] or "electropermeabilizaiton" [26]. Since the exact molecular mechanisms of the phenomena are not known both terms are accepted in this field.

A critical concept for understanding of cell electropermeabilizaiton is an induced transmembrane potential $\Delta\varphi_m$. It is

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thought that the control over $\Delta\varphi_m$ by electric fields causes permanent or temporary cell membrane permeabilization. Schwan [27] showed that for a single spherical cell, the $\Delta\varphi_m$ depends on the angle θ between the external field E and the radius vector r on the membrane surface as follows:

$$\Delta\varphi_m = -1.5 \cdot E \cdot f(\sigma) \cdot a \cdot \cos\theta$$

where $\Delta\varphi_m$ is the potential difference at the specific location on the membrane, E is the applied external electric field, a is the cell radius, θ is the angle of the radial direction vector ($\theta = 0$ and $cos\ \theta = 1$ when the vector coincides with the direction of the electrical field). $f(\sigma)$ is an explicit function of the electrical conductivities of the suspending medium, the cell interior and the cell membrane [27].

The important outcome of the relation aforementioned is that $\Delta\varphi_m$ strongly depends on the position along the cell membrane, through the cosine term in the equation. The maximum $\Delta\varphi_m$ is located at the poles of the cell, where the cosine term has a maximum. Experiments with voltage sensitive dyes confirmed that the maximum $\Delta\varphi_m$ drop appears on cell poles that face the electrodes [28]. Numerical solutions for $\Delta\varphi_m$ for various nonspherical cells were developed in [30].

The first theoretical models that proposed an electromechanical mechanism for membrane breakage [31], could not account for the pulse duration dependence of the critical voltage needed for membrane permeabilization [32], or the dependence of the model membrane lifetime on the total membrane area [33]. The currently accepted, transient aqueous pore hypothesis, reviewed in [34], suggests that the process of electroporation is related to formation of nanoscale pores (defects) in the cell membrane. This hypothesis proposes the following steps in the electroporation phenomena: 1) charging and polarization of the membrane (charging time in the range of a few μs); 2) membrane structure destabilization and creation of hydrophobic pores; 3) pore radius growth and stabilization; and 4) pore resealing and cell survival, or cell death because of the large defects [26], [34], [35]. Based on these theoretical models, the number, density, radius, spatial distribution, and lifetime of pores can be calculated for different cells and tissues [36].

Although the membrane pores have not been observed experimentally yet, multimolecular dynamics stimulations provide additional insights on the electroporation processes. Molecular dynamics stimulations of lipid bilayers propose the following cascade of events for pore formation. First, within a very short (nanosecond) time scale, a defect in the membrane is formed by the appearance of water fingers that enter from the lipid headgroups/water layers in the lipid core. Second, if the threshold $\Delta\varphi_m$ is maintained an adequate amount of time, the fingers span the entire hydrophobic core, and thus they form hydrophilic pores that are later stabilized by lipid headgroups (see Fig. 1). Finally, when the field is switched OFF, the pores reseal [37], reviewed in [38].

II. Experimental Models

The first work on NTIRE was published by Davalos *et al.* in [24]. Using mathematical models, the authors predicted the possibility to nonthermally ablate clinically relevant volumes of tissue by NTIRE [24]. This theoretical work was later confirmed by experimental evidences on cell culture [39], small [40], and large animal models [41].

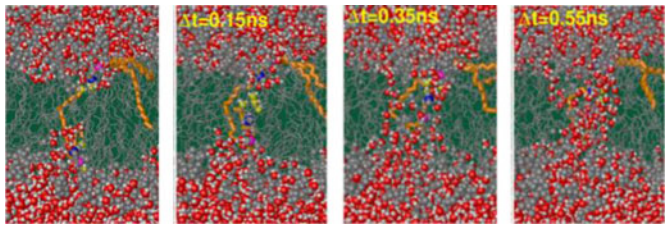

Fig. 1. Primary electroporation events. Snapshots of the electropore formation. Lipids and water molecules guiding the initial steps are highlighted. The lipid headgroups are shown as gray balls, water oxygen as red balls, and lipid tails as sticks. Water molecules forming the initial membrane-spanning water file are colored yellow. Image adopted from [37] with permit.

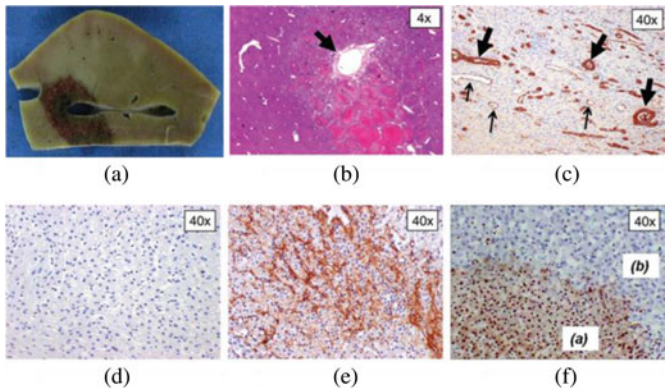

Fig. 2. NTIRE histopathology. (a) Gross pathologic-sectioned specimen of NTIRE-ablated swine liver shows areas of discoloration caused by ablation. (b) Preserved large vessel (arrow) in area of NTIRE ablation stained with H&E. (c) Numerous capillaries (thin arrows) and bile ducts (thick arrows) stained with vWF revealing structural preservation. (d) Normal liver tissue shows mild vWF staining of vessels and bile ducts. (e) Ablated zone shows markedly increased vWF positive staining along the sinusoids, as well as staining of vessels and bile ducts. (f) Ablated zone *(a)* shows increased apoptotic markers in TUNEL assay, compared with normal liver *(b)*. Images adopted from [4] with permit.

Results from various animal species, including mice [42], rat [5], rabbit [43], dog [44], goat [45], and pig [46], further confirmed that NTIRE can ablate clinically relevant tissue volumes nonthermally. Successful ablations were reported for brain [44], [47], [48], blood-brain barrier [47], prostate [50], [51], heart [52] pancreas [53], [54], small intestine [5], lung [55]–[57], kidney [58]–[62], nerves [8], [63] cutaneous tumors [6], [42], uveal melanoma [64], blood vessels [3], [65], bone [66], [67], head and neck cancer [68], and liver and liver tumors [4], [43] [45], [46], [69]–[74]. Although the parameters used for tissue ablation vary somewhat among these studies, the ranges used include field intensities of 1000–2500 Vcm^{-1}, 70 μs–20 ms pulse duration, and 1–90 pulses delivered at 0.1–1 Hz.

A distinguishing property of NTIRE is the damage to the cell membrane only; tissue architecture—scaffold, large blood vessels and bile ducts—appears to be spared (see Fig. 2) [3]–[5], [8], [63], [65]. Moreover, metabolic function of treated organs was only temporary affected, as reported for pancreas and

liver [53]. The preservation of tissue architecture is an important and unique property of NTIRE ablation method for it probably contributes to the reduced scarring observed in [3], [5], [41], and [65].

The molecular mechanisms of cell death after NTIRE are still not clear. Though histopathological analyzes imply that both necrosis and apoptosis [see Fig. 2(f)] are involved [4], [6], the reports on pathways that lead to cell death are contradictive. For instance, Guo *et al*. [72] reported on extensive caspase-3 activation 24 h after the NTIRE of rat hepatocellular carcinoma, suggesting apoptosis. In another work, however, Jose *et al.* [53] did not detect any caspase-3 positive cells in the treated area of pancreatic carcinoma. The latter results suggested only necrotic cell death [53]. The authors argue that there may be differences based on the tumor model used [53]; however, the question whether NTIRE effects on the cell membrane serve as an final event, or whether it also induces long-term programmed cell death is still open for further studies.

Finally, it is important to point out that even at the single cellular level in culture, NTIRE cell death is a statistical phenomena [39], [75], [76]. The cell death mechanism is even more complicated in the living tissues because of the organism's complex response to injury. Only a limited number of works deal with the inflammation response to NTIRE. Al-Sakere *et al*. [7] concluded that NTIRE does not induce a substantial infiltration of immune cells into the treated tissue; thus, the immune response is not instrumental for successful NTIRE. In contrast, Onic *et al*. [50] observed a significant immunologic reaction in the lymph nodes draining the prostate area ablated by NTIRE. Li *et al*. [77] recently reported that NTIRE significantly modified the cellular immune response, in comparison with surgical resection, in the osteosarcoma rat model experiment. Moreover, it appears that the immunological response is essential for the NTIRE ablation that leads to tissue decellularization [5], [78].

III. Clinical Data on Irreversible Electroporation

Following the encouraging results in animal models, human studies have started. The principals for the design of clinical NTIRE device appeared in [79], and the first NTIRE application in humans was performed on the prostate cancer [80]. In this study, 16 patients with prostate cancer were treated by four round square array electrodes separated 1-1.5 cm, 90 pulses, 70–100 μs duration, with applied voltage 1500 V, delivered at 10 Hz. Postoperative biopsies from the area of previously known cancer in 14 patients showed no evidence for cancer. One patient with a negligible prostate-specific antigen (PSA) refused a postoperative biopsy. In an additional patient, though the treated area was cancer free, a micro focus of cancer was found outside the treated area [80]. Furthermore, Doppler ultrasound demonstrated intact and functioning micro- and macrovasculature at the treated region. No complications were reported in that study.

Subsequent studies investigated the safety and efficacy of NTIRE treatment of pancreatic adenocarcinoma [81]–[83] renal cell carcinoma [84], [85], lung malignant tumors [85], [86], and hepatic malignant tumors [85], [87], [88]. The special anesthesia aspects of the NTIRE ablation are discussed in [89].

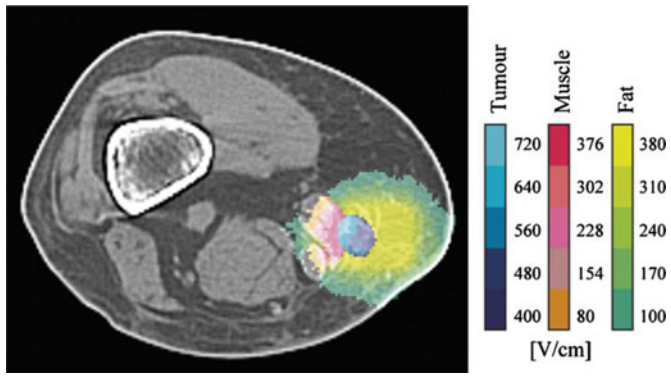

Fig. 3. Cross section plot of the integrated tissue electroporation treatment planning methodology. The slice shows a combination of a patient melanoma CT image with the predicted electric field distribution. NTIRE ablated volume is delineated from the electric field distribution. Image adopted from [97] with permit.

These studies concluded that the NTIRE procedure was safe in those studies. Cardiac arrhythmias that arose during NTIRE in several patients were managed by electrocardiographically synchronized delivery of pulses. The efficacy of the procedure, however, varied between the studies and treated organs. In pancreatic and prostate cancer 100% success in tumor ablation was reported in [80]–[82]; however, none of the treated lung tumors were successful ablated up to date [85], [86]. In liver, the complete response varied from 50% to 98.1% in different studies [85], [87], [88]. The clinical reports concluded that additional large-scale trials are required to determine the procedure safety and efficacy.

IV. Mathematical Modeling for the Treatment Planning

The treatment planning is an essential procedure for the successful NTIRE therapy. The ultimate goal of the treatment planning is to model electrode position and number, electric field amplitude, pulse duration, number and frequency to nonthermally ablate only the targeted tissue. It is interesting, the very first paper on NTIRE was a theoretical study which with the use of finite-element modeling (FEM) predicted nonthermal tissue ablation volumes by pulsed electric fields [24].

The current treatment planning models focus on: 1) electrode position, to cover the target tissue by electric fields and spare nontarget tissue [41], [90], [91]; 2) electric field protocol optimization, to delineate the thermal damage from NTIRE [24], [92]–[96]; and 3) integration of mathematically derived treatment planning with diagnostic imaging [48], [97]. The example of the integrated planning model which includes medical image analysis, FEM of electroporation, visualization and a genetic algorithm for optimization of a number of electrodes, and applied pulsed-electric field protocol appears in Fig. 3 [97]. Here, the authors show the computed tomography (CT) scan of melanoma metastases, combined with the optimized position of four electrodes and the spatial electric field distribution. The combined approach allows effective planning and optimization of the NTIRE procedure.

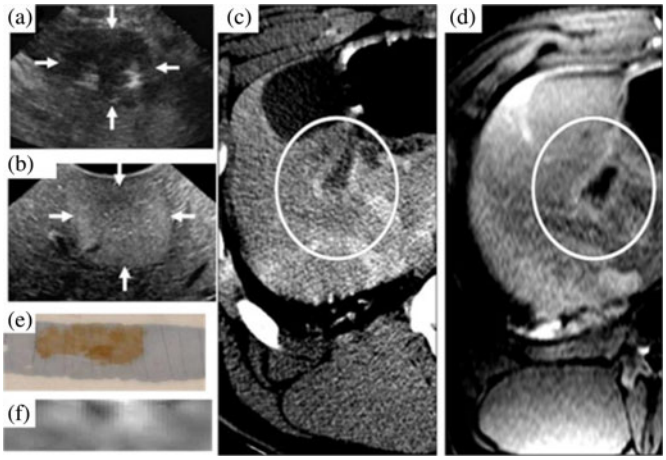

Fig. 4. NTIRE imaging methods. (a) Real-time US images from ablation of a normal pig liver. The ablated zone is well visualized immediately after the procedure (arrows). (b) US image, 24 h post ablation. (c) Contrast-enhanced CT scan obtained in venous phase of the ablated liver. (d) Contrast-enhanced fat-saturated T1-weighted gradient-echo MR image of normal pig liver ablation. (e) Diaminobenzidine stain of rat liver ablated by NTIRE. (f) EIT as measured immediately after the pulse. Images adopted from [4] and [109] with permit.

V. Imaging

An important clinical advantage of NTIRE is the ability of physicians to use existing imaging methods, such as an ultrasound (US), CT, magnetic resonance imaging (MRI), electrical impedance and optical measurements, for procedure guidance.

US has been used in animal and clinical studies for real-time electrode positioning, immediate and posttreatment imaging, [4], [41]–[46], [69], [70]. The US findings after NTIRE are dynamic [see Fig. 4(b)], and are tightly correlated with the histological observations. In liver, NTIRE ablated region is immediately visualized as a hypoechoic area [see Fig. 4(a)], [70]; however, already after 90–120 min the external hyperechoic rim appears, probably due to hemorrhagic infiltration [4]. The full transition of hypoechoic area to hyperechoic was observed 24 h after treatment [4]. The tightest correlation was found between the size of histological findings of ablated tissue with the hyperechoic rim observed 90–120 min after the NTIRE treatment [69].

Noncontrast and contrast-enhanced CT is used in animal experiment and patient care for diagnostics, treatment planning, electrode positioning and posttreatment evaluation of tissue ablation, and tumor recession [4], [43], [55], [81], [82], [88]. NTIRE ablation of liver results in hypoattenuating area with a hyperattenuating rim in the ablated area two days after the procedure [see Fig. 4(c)], [4]. Immediate changes in the tissue structure following three weeks evolution were reported in the pig lung NTIRE study [55]. In this study, however, the authors mentioned that immediately after the treatment the images varied in appearance, making the measurement of the ablation zone difficult and that a there is a need for systematic studies on NTIRE tissue changes as observed by CT.

MRI techniques are often used for detecting changes in tissue structure and functionality assessment after NTIRE in animals and human subjects, [4], [42]–[44], [47]–[49], [58], [82], [88], [98]–[100]. Systematic studies on NTIRE ablation of potato [101], rat blood-brain barrier [49], murine tumors [42], rat liver [99], and rat brains [98] demonstrated dynamic behavior of various MRI sequences of the ablated tissue.

Studies show that the ablated volumes detected by T1-weighted (T1W), [see Fig. 4(d)], transverse relaxation time (T2) and T2-weighted (T2W), FLAIR and diffusion-weighted magnetic resonance imaging (DW-MRI) for apparent water diffusion coefficient (ADC) are similar to the volumes detected by histological observations. Moreover, T1-Thrive5-3D-GE urogram scans were used to validate the functionality of NTIRE ablated swine kidney together with intravenous urography [58]. Nevertheless, the specific useful MRI sequence depends on the ablated tissue. Although ADC allowed the visualization of early and rapid changes in the treated murine tumors [42], no significant changes in ADC were observed during the blood-brain-barrier NTIRE ablation up to 30 min posttreatment [49].

Though effective in the standard clinical facilities, CT/MRI imaging demands expensive imaging equipment, often not available in developing countries or in rural areas. Monitoring the change in tissue electrical properties is a convenient method for low-cost tissue diagnostics [102]–[104]. Both reversible electroporation and NTIRE lead to immediately detectable tissue impedance decrease used for treatment planning and procedures outcome assessment [102]–[108]. The 2-D reconstruction of tissue impedance measurement, a method called electrical impedance tomography (EIT), allows real-time monitoring of tissue NTIRE [109], [110]. The increase in the treated tissue conductivity is clearly seen in the reconstructed images (area with the increased brightness) and is correlated with histological staining [see Fig. 4(e) and (f)] [109]. In addition to EIT, magnetic resonance electrical impedance tomography and current density imaging (CDI) were proposed for measurement of currents and electric fields distribution in tissue during and after NTIRE [111], [112]. Though promising, the *in vivo* results for MRIET and CDI have not been published yet.

Optical whole tissue imaging is a convenient tool to monitor treated cells fate. To date, bioluminescence and fluorescence have been used in the NTIRE animals studies [53], [113]. In the first study, luciferase-expressing cancer cells were injected into mice to induce florescent pancreas cancer model [53]. The bioluminescent emission was monitored up to 90 days to follow tumor progression. Four days post NTIRE, lower emission was monitored in the treated animals than in the control. Moreover, at the end of the experiment complete tumor eradication was achieved in 25% of the mice [53]. In the second work, green-florescent-protein (GFP) was injected in to pig liver before NTIRE. Six hours after NTIRE ablation GFP expression was observed in the treated liver area [113] emphasizing the affected area. The authors suggested that reversible electroporation, which facilitates gene transfer, occurred in the remote tissue areas, where the electric field strength was lower than in the center. An additional explanation is that IRE affected cells did not die immediately, but in a long-term apoptotic process. Thus, the affected cells expressed GFP before they die [113].

VI. Future Trends and Challenges

The ability of NTIRE to ablate tissue nonthermally, while sparing the major tissue architectural components, accessible

imaging and fast patient recovery generated a great deal of interest in basic and translational research in the past several years. This resulted in emergence of data across species, from various tissues, and from various clinical applications. As mentioned previously, encouraging results were reported on animal models and in several clinical trials on pancreas and prostate ablation. The failure, however, to ablate lung tumors and the somewhat contradictory results with regard to cell death mechanisms after NTIRE remains to be clarified and overcome.

To use the NTIRE efficiently, we need to understand the basic mechanisms of damage and regeneration on molecular, cellular, and tissue level. Presumably, the molecular understanding will emerge from advanced molecular dynamics stimulations. Investigation of the impact of NTIRE on homogeneous and heterogeneous tissues will address the important question of selectivity, and inform whether NTIRE can be a selective ablation method for various cell types. In addition, further studies on the role of the immune system response to NTIRE induced damage are needed, and will presumably provide answers concerning *in vivo* mechanisms of cell death and regeneration. Furthermore, the supposition that the preservation of the tissue architecture generates full recovery demands additional research.

An additional challenge is to eliminate NTIRE induced muscle contraction without using total body muscle paralyzes agents [71], [91]. Solving this problem will significantly simplify the use of NTIRE for global medicine, including its use in developing countries. Moreover, further integration of imaging for treatment planning and using optimization algorithms for electrode positing, and real-time image guided procedure control are needed. This integration will provide precise treatment with fewer side effects. Systematic studies, however, are required to characterize NTIRE induced dynamic changes in tissues for various imaging methods.

Only few clinical data have been published so far. The encouraging results from success in ablation of prostate and surgically unresectable pancreatic cancer reveal the clinical advantages of NTIRE. Though in the reported studies, the NTIRE procedure was recognized as safe, additional large-scale translational research is needed to show how NTIRE will affect clinical outcomes for various clinical conditions. The long-term observations on tumor recurrence and tissue repair will contribute to the wide acceptance of NTIRE into medical practice.

VII. Conclusion

NTIRE is an emerging medical application at the interface of engineering, basic life science, and medicine. Current knowledge of NTIRE suggests that it has vast potential for important many medical applications. Although a full understanding of the fundamental mechanisms of NTIRE on the cell membrane, tissue responses, and regeneration has yet to be developed, its use in basic science and therapy will no doubt continue and strengthen.

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