HeartRhythm

The Official Journal of the Heart Rhythm Society, The Cardiac Electrophysiology Society, and The Pediatric & Congenital Electrophysiology Society

Heart Rhythm Society SM

Pulsed field ablation: Disrupting technology in cardiac electrophysiology

Damijan Miklavčič, PhD,[1] Lea Rems, PhD,[1] Matevž Jan, MD, PhD,[2] Bor Kos, PhD[1]

ABSTRACT

Pulsed field ablation (PFA) is being adopted as a safer and more efficient alternative to conventional thermal ablation methods for the treatment of cardiac arrhythmias, particularly atrial fibrillation. In this review, we examine the basic biophysics of PFA, focusing on electroporation at the membrane, cellular, and tissue levels to provide mechanistic explanations for the observed clinical outcomes. We analyze both the benefits and limitations of the nonthermal, tissue-selective nature of PFA, examine adverse events, and emphasize the need for standardized comparisons among different manufacturers' systems. Drawing on decades of electroporation research in other biomedical fields, we suggest that deeper scientific understanding is key to optimizing PFA technology, improving long-term outcomes, and maintaining its strong safety profile. Open questions and future directions for clinical translation and refinement of the procedure are also discussed, considering both atrial and ventricular ablation.

KEYWORDS Pulsed field ablation; Electroporation; Cardiac ablation; Limitations; Tissue selectivity; Adverse events of PFA

(Heart Rhythm 2025;■:1–12)

Pulsed field ablation (PFA) is increasingly replacing conventional thermal ablation methods in the treatment of atrial fibrillation (AF).[1,2] PFA is also being actively explored for the treatment of ventricular tachycardia (VT).[3–5] Compared with thermal methods, PFA is safer, more time effective, and comparably efficient.[6,7] Although superior (ie, 100%) acute pulmonary vein isolation (PVI) is usually achieved with PFA, long-term treatment success in terms of freedom from AF and reduction of AF burden still needs improvement. It is currently also not yet possible to distinguish between long-term failure owing to failure to ablate the target completely, for example, achieve transmural and circumferential ablation in PVI,[8] and failure owing to unknown mechanisms and origins of the arrhythmia. Furthermore, owing to the mostly nondisclosed waveforms of current PFA systems, it is difficult to make laboratory comparisons among the different systems, and clinical comparisons remain scarce.

The safety of PFA with various systems has been demonstrated in preclinical studies and the first clinical trials. This experience has been largely confirmed in reports from the real world.[9,10] The total number of energy delivery-related adverse events is consistently less than 1%, without the occurrence of atrioesophageal fistula, long-term phrenic nerve palsy, or pulmonary vein stenosis. With the spread and use of PFA beyond PVI, new adverse events have been identified: hemolysis, coronary artery spasms, and transient phrenic nerve paresis. In addition, cerebrovascular events (silent cerebral lesions [SCLs], transient ischemic attacks [TIAs], and strokes) have been reported but do not have significantly different rates than in thermal procedures.

PFA was introduced as a nonthermal and tissue-specific/selective ablation method based on electroporation. Electroporation has been used in biomedicine for drug and gene delivery, biotechnology, and food processing for decades.[11–13] The wealth of knowledge accumulated in these areas can help explain many of the observed adverse events, while also improving our understanding and aiding in the development of PFA procedures and devices. Meaningful comparison of different devices and treatment outcomes, as well as the development of periprocedural markers, will only be possible through a deeper understanding of PFA mechanisms. Ultimately, comprehending PFA, including the

From the [1]Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia, and [2]Department of Cardiovascular Surgery, University Clinical Medical Centre, Ljubljana, Slovenia.

https://doi.org/10.1016/j.hrthm.2025.12.022

1547-5271/$-see front matter

limitations of its "non-thermal" nature and how the selectivity of cardiac tissue ablation can be utilized, is critical for maximizing the potential of this new ablation modality.

This review describes the phenomenon of electroporation at the membrane, cellular, and tissue levels and outlines the biophysics of atrial and ventricular ablation, as currently understood. It offers mechanistic explanations for acute vs long-term success and adverse events, which will hopefully help maintain the superior safety and efficiency of PFA for treatment of cardiac arrhythmias.

Electroporation at the membrane and cellular levels

The fundamental mechanism underlying PFA is cell membrane electroporation.[14] When a cell is exposed to an external electric field, for example, by applying electric pulses (Figure 1A), the cell membrane becomes charged and a voltage is induced across the membrane (Figure 1B). From an electric perspective, the cell membrane behaves like a thin dielectric sheet surrounded by electrolyte (both intracellular and extracellular solutions), essentially functioning as a capacitor. The induced transmembrane voltage is proportional to the electric field strength and cell size, depends on cell orientation, and varies with position on the membrane.[15] When this induced transmembrane voltage is within physiological values ($\leq$100 mV), it causes stimulation of excitable cells; that is, it triggers an action potential and calcium release and causes muscle contraction (Figure 1C). However, when the transmembrane voltage reaches supraphysiological values (typically several hundred millivolts), it causes structural and chemical changes in the membrane that increase the membrane's permeability to ions and molecules (Figure 1D). These changes include the formation of aqueous pores in lipid domains and membrane proteins, as well as lipid oxidation and the formation of permeable defects in oxidized membrane lesions.[16–19] The formation of these pores and defects results directly from the strong electric field within the membrane. A transmembrane voltage of 0.5 V across a 5 nm thick membrane results in an electric field strength on the order of 10^8 V/m—sufficient to break most dielectric materials, including a lipid bilayer/cell membrane.

The resulting increased membrane permeability increases the transport of ions and molecules across the membrane in both directions (into and out of the cell), which disrupts the ion gradients, increases the intracellular calcium concentration, and causes adenosine triphosphate and other substances, such as damage-associated molecular pattern molecules, to leak out of the cells (Figure 1D).[20–22] The downstream effects are numerous and include disruption of the cytoskeleton network and decreased mitochondrial membrane potential.[23,24] Therefore, electroporation can be regarded as a form of cell injury. Cells have their own mechanisms to repair such injuries and can therefore recover homeostasis and survive electroporation, a process known as reversible electroporation. However, if cells cannot repair the damage, they die—a process known as irreversible electroporation. Irreversible electroporation typically occurs at higher electric field strengths and/or a greater number of pulses than reversible electroporation, given that it is associated with higher transmembrane voltages, larger electroporated membrane areas, greater transmembrane transport, and therefore greater disruption of homeostatic balance.

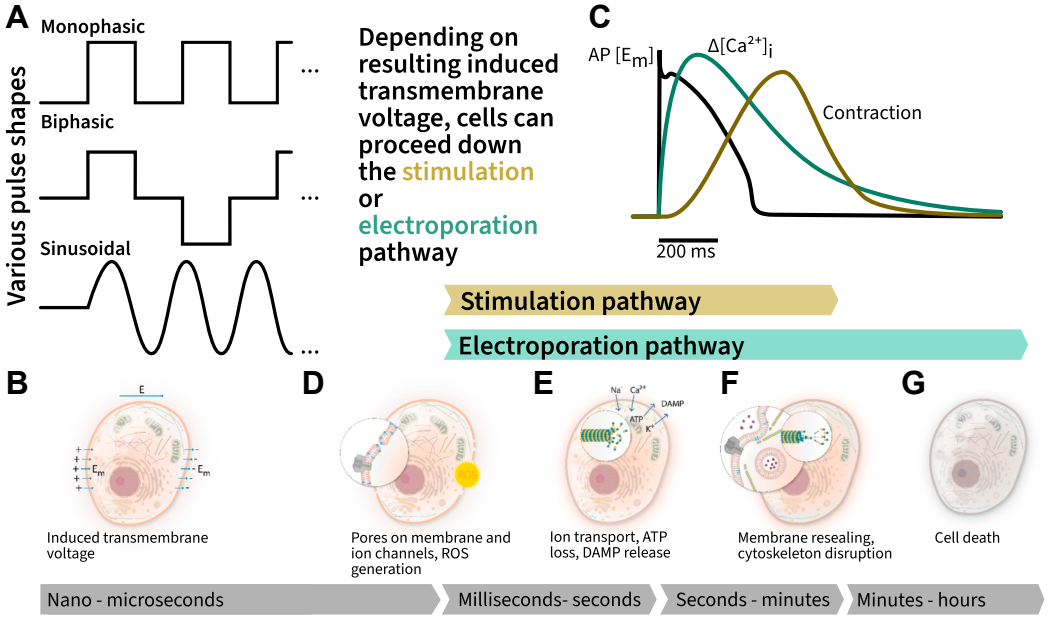


Figure 1
Cell response to pulsed electric fields. **A:** Cell can be exposed to pulses of different shapes and durations. **B:** When exposed to an electric field, an induced transmembrane voltage will be established that will, **(C)** at lower values, trigger physiological response, ie, action potential and contraction in muscle cell, and **(D)** at higher values cause electroporation with **(E and F)** downstream effects potentially leading to **(G)** cell death. AP = action potential; ATP = adenosine triphosphate; DAMP = damage-associated molecular pattern; ROS = reactive oxygen species.

Cell death can be necrotic at higher electric field strengths and apoptotic, necroptotic, or pyroptotic at lower electric field strengths.[25] It is not yet clear whether there are any significant differences in the clinical implications of the different cell death pathways listed earlier, especially because some combination of cell death pathways is likely to occur with every PFA application. The main differences are likely in their immunogenicity, given that electroporation-mediated cell death can trigger an immune response.[26] It is important to note that cell death does not necessarily occur immediately—cells can successfully reseal their membranes but still die hours later.[27]

The disruption of ionic gradients also affects cellular excitability (Figure 2).[28–30] An increase in membrane permeability results in increased membrane electric conductance. This increase is nonselective, and therefore, electroporation adds an additional leak current across the membrane that bypasses currents through ion channels and pumps.[31] Depending on its magnitude, this leak current can alter the shape of action potentials or cause sustained membrane depolarization and stunning—a temporary inhibition of action potentials.[32] If electroporation is reversible, the cell reseals its membrane within seconds to minutes after exposure to the pulses, allowing a gradual recovery of excitability. The rapid increase in membrane conductance after PFA and subsequent resealing may explain the immediate disappearance of intracardiac electrograms (iEGMs) and their (partial) recovery within minutes.[33] Action potentials after this recovery phase may still be impaired to some extent for a longer period (after membrane resealing itself is complete), if electroporation has resulted in damage to the ion channels.[17] Electroporation can also result in disruption of intercellular connections (gap junctions),[34] which can affect the propagation of action potentials in excitable tissue.[35] Both—the effects of electroporation on ion channels and gap junctions—require further research. Electroporation can also alter transcriptomic and proteomic profiles in both reversibly electroporated cells and cells undergoing cell death,[36] which may have further effects on cellular function. The induced transmembrane voltage—the prerequisite for membrane electroporation—also depends on the duration of individual electric pulses applied in the waveform. If the pulse duration exceeds the characteristic membrane charging time (on the order of 1 μs for cardiomyocytes), the membrane can fully charge and reach its maximum value. For shorter pulses, the membrane does not fully charge

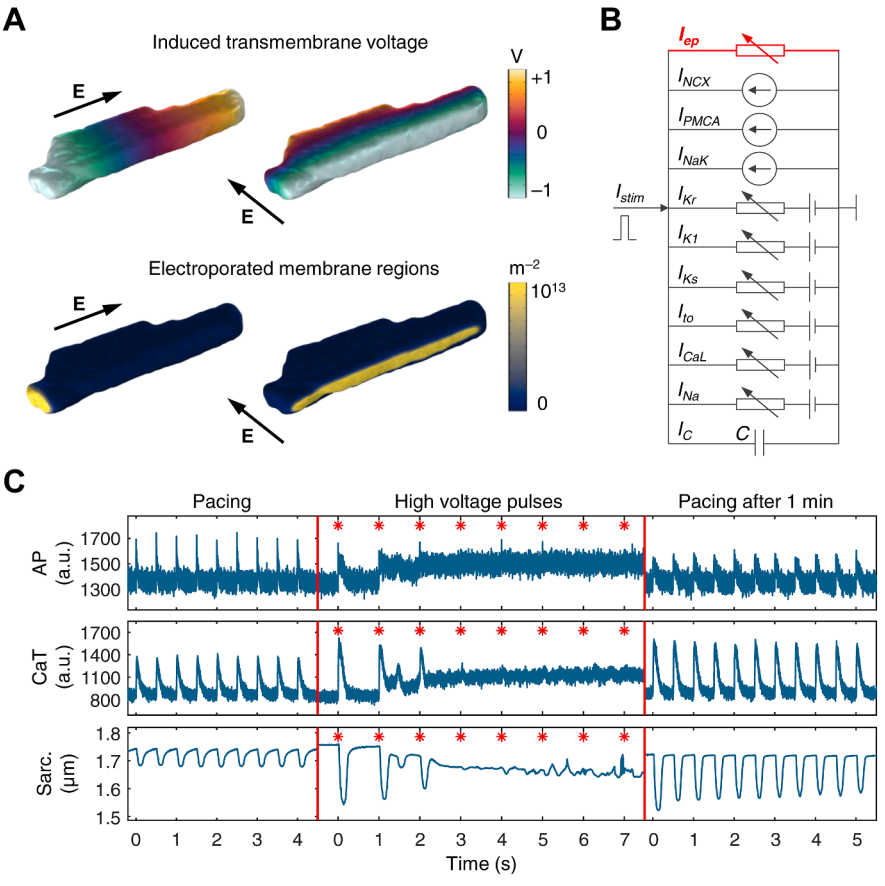


Figure 2

Electroporation at the cellular level in cardiac pulsed field ablation. **A:** The profile of the induced transmembrane voltage depends on cardiomyocyte orientation in the electric field. Electroporation correlates with the membrane regions where the induced transmembrane voltage reaches the highest absolute values. **B:** Equivalent electric circuit representing the cardiomyocyte membrane with its main ion channels and pumps. Electroporation adds a nonselective leak current (I_{ep}) to the membrane. **C:** Response of an adult rat cardiomyocyte to high-voltage pulses (indicated by *asterisks*). When electroporation occurs, the action potentials (APs), calcium transients (CaT), and sarcomere shortening (Sarc.) become perturbed; however, a cardiomyocyte can recover from such electroporation.

before the end of the pulse and therefore reaches a lower transmembrane voltage value. However, this can be compensated for by increasing the applied electric field strength, that is, using a higher pulse amplitude. Therefore, waveforms with shorter pulse durations typically require higher electric field strengths to achieve electroporation. In addition, the induced transmembrane voltage depends on cell orientation in a pulse duration-dependent manner. For pulses longer than about 10 μs, cells oriented with their long axis parallel to the electric field are more sensitive, whereas for submicrosecond duration pulses cells oriented perpendicular are more sensitive. For pulses with duration roughly in the range of 1−10 μs (depending also on the type of cell), the orientation sensitivity becomes less important and cells are more similarly electroporated regardless of their orientation.[37,38]

Electroporation at the tissue level

An electric field of sufficient strength to cause electroporation in the tissue is usually established by delivering electric pulses through electrodes. In PFA, this is achieved by placing the ablation electrode adjacent to the target substrate to be ablated (Figure 3A). During pulse application, the electric field and current density are highest in the immediate vicinity of the electrodes on the catheter but decrease rapidly with increasing distance from the catheter surface (Figure 3B). The highest current density is expected at sharp "edges," either at physically sharp edges of metal electrodes or at sharp edges that form at the junction of metal electrode and insulation. A significant increase in temperatures can be expected there.[39,40] Even if this increase is limited to a small volume, it can lead to thermal coagulation necrosis in the tissue, and also an increase in temperature in the blood. Although the latter is short lived, it should not be neglected because it may be sufficient to cause protein denaturation and clot formation. In addition, electrolysis, degassing, and boiling of water can cause bubbles to form. The localized overheating of the blood around the catheter is highly dependent on the waveform and in particular on the amount of energy delivered in a unit of time. This local overheating (even at temperatures below boiling) can lead to clotting and bubble formation, which in turn can result in thromboembolic events such as SCLs, TIA, and stroke.[41]

At a greater distance from the catheter in the tissue, there is a volume in which the cells die with some delay. For PFA, this is the desired outcome and the end goal of the treatment—to destroy myocardium that is propagating the arrhythmogenic signals and therefore prevent their conduction. In older literature, this is typically referred to as irreversible electroporation—now PFA (Figure 3B). The minimum electric field at which this occurs is typically named lethal electric field threshold (LET).[42]

Surrounding the volume of cell death is a region where stunning, that is, reversible electroporation takes place (Figure 3B and as described in the section earlier). This still requires some degree of electroporation to produce the membrane damage that leads to the nonselective leak currents that inhibit normal cell repolarization. Stimulation of excitable cells can occur at even lower electric field strengths, so the volume at which this can occur is even greater.[32,43,44] Recently, a preclinical study showed that it is also possible to use a single monophasic 10 μs pulse to achieve cardioversion and termination of AF and atrial flutter.[45]

Electroporation causes various changes in the tissue. During the pulse application, the conductivity of the tissue is temporarily increased by a factor of up to 3 compared with the initial conductivity.[46,47] This is because electroporation forms additional conduction pathways through the

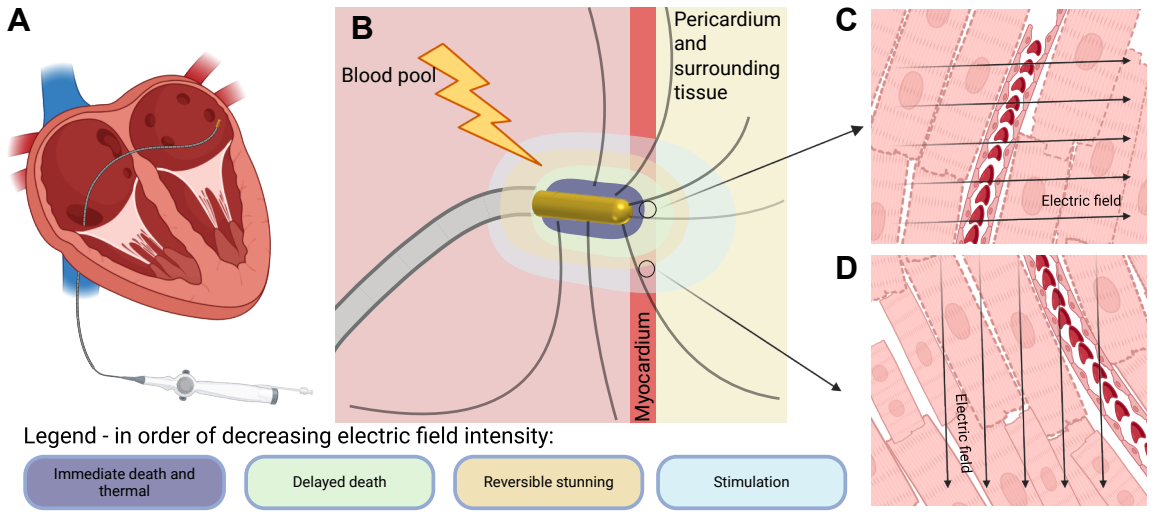


Figure 3
Electroporation at the tissue level in cardiac pulsed field ablation. **A:** Example of placing a monopolar focal ablation catheter in the left atrium. **B:** Electric field in tissue surrounding the catheter—blood, myocardium, and pericardium and surrounding tissue. The electric field is illustrated with nested volumes of different effects. **C:** Electric field in perpendicular orientation relative to the long axis of cardiomyocytes. **D:** Electric field parallel with the long axis of the cardiomyocytes. Created in BioRender. Kos et al.[41] https://BioRender.com/oma0r8h.

cell membranes, which otherwise represent an obstacle to the flow of current. The increase in conductivity also depends on the duration and shape of the pulse waveform.[48,49] For the myocardium, this conductivity increase factor is 2.6 for 100 μs pulses and slightly less—1.8 for the short biphasic pulses used with the circular array catheter.[42] This change in conductivity affects the distribution of the electric field in the tissue and also the shape of the lesion. The conductivity of the blood is less affected, mainly because the volume fraction of cells in the blood is much smaller, but also because the cells have a much smaller diameter than cardiomyocytes. Consequently, the threshold value of the electric fields at which electroporation of small blood cells occurs is expected to be much higher.[50] After pulse application, conductivity generally returns to pretreatment values, but measurements taken shortly after the end of pulse delivery still show an increase from baseline.[47] This can be attributed in part to residual heat from the pulse applications, given that all tissues have a positive temperature coefficient for conductivity in the range of 1%–2%/°C,[51] and in part to the edema caused by the electroporation itself.[52]

Endothelial cells of the capillaries and also of the larger blood vessels are also affected by electroporation, making the walls of all blood vessels more permeable (Figure 3C and 3D).[53] The direction of the electric field relative to the cardiac muscle fibers (Figure 3C and 3D) also affects the shape of the lesions, partly owing to the different susceptibility of cells to electroporation as a function of their orientation,[37] but also owing to the anisotropic conductivity of the myocardium.[42,54]

Stimulation induced by electric fields further from the electrodes (Figure 3B) can elicit various physiological responses such as neuromuscular capture, pain,[55,56] stimulation of neurons of ganglionic plexi, and contraction of smooth muscles. Contraction of the esophagus and its retraction during PFA have recently been suggested as one of the contributing mechanisms to functional sparing of the esophagus.[57] Given that the esophagus can be stimulated from PFA applications, it is also reasonable to assume that stimulation of smooth muscles can play a role in the spasm of coronary vessels, particularly because it has been shown that blood flow can be dramatically reduced already after just a few pulses being delivered.[58]

Although there were some initial claims that PFA is selective in killing only cardiomyocytes based on in vitro data,[35,59,60] the differences in LET of different tissues as determined in in vivo experiments are much smaller and the observed selectivity may rather stem from the different capacity for regeneration that different tissue types have.[61]

Determinants of lesion size and shape

Beyond pulse duration and amplitude, biological effects of electroporation also depend on the number of applied pulses in pulse trains, pulse shape (monophasic vs biphasic, rectangular vs exponentially decaying), pulse repetition frequency, etc.[62–64] All these pulse parameters must be considered and carefully selected to achieve the desired electroporation effect while minimizing side effects, such as excessive heating, bubble formation, and neuromuscular capture.[41]

While keeping all other pulse parameters constant, increasing the number of pulse trains reduces the LET in tissue. Although several different functions for empirically fitting this dependence have been evaluated,[64,65] a recent in vivo study found that the power function offers a good fit of volume of lesions in swine ventricular myocardium.[52] This function illustrates that there are diminishing returns with increasing the number of pulse trains. Consequently, the reduction in LET that can be achieved with an increased number of trains still cannot overcome the rapid decrease in electric field strength with distance from the electrodes, meaning that the practically achievable lesion size with a given waveform and catheter combination is limited.

Lesion shape is also affected by the catheter geometry and the choice of vectoring. Bipolar catheters have electric fields that are very high near the surface of the electrodes, but decrease rapidly with the distance from the electrodes.[66] In contrast, unipolar catheters cause the electric field in a larger volume and also tend to achieve deeper focal lesions.[52,67,68] However, a direct comparison of the same catheter and waveform did not show much difference between bipolar and monopolar configurations.[69] Furthermore, it is important to note that LET is a simplification of the complex biological process of cell death from a relatively simple electric field distribution and that it sometimes fails to accurately reflect the size and shape of the lesions.[70,71]

Table 1 Different effects of various ablation energies on tissue and cells

Ablation method	Radiofrequency ablation (heating)	Cryoablation (freeze-thaw)	Pulsed field ablation (electroporation)
Energy delivery and propagation	Volumetric heating and thermal diffusion	Thermal diffusion	Electric field effect and volumetric heating
Cell damage	Denaturation of proteins	Crystal formation—cell membrane damage; osmotic imbalance	Cell membrane and membrane protein damage
(Micro-) vasculature	Highly thrombogenic, indiscriminate thermal damage	Clogging and damaging of the microvasculature	Transient reduction of blood perfusion
Extracellular matrix	Coagulation	Preserved	Preserved
Healing	Delayed, from the periphery	Delayed, from the periphery	Enabled/facilitated

The lesion achieved in tissue develops and matures over the course of several weeks.[52,72] Given that the mechanisms of causing cell death in PFA are different from the mechanisms in thermal ablations (Table 1), the lesion development and maturation are also different and different healing dynamics can be expected.[61,73] It thus remains to be confirmed if late gadolinium enhancement (LGE) cardiac magnetic resonance imaging (MRI) can be used in the same way and at the same times as after thermal ablation. In addition, it remains to be established whether LGE can be used to assess transmurality of lesions and pulmonary vein reconnections.[74,75]

Waveform and catheters

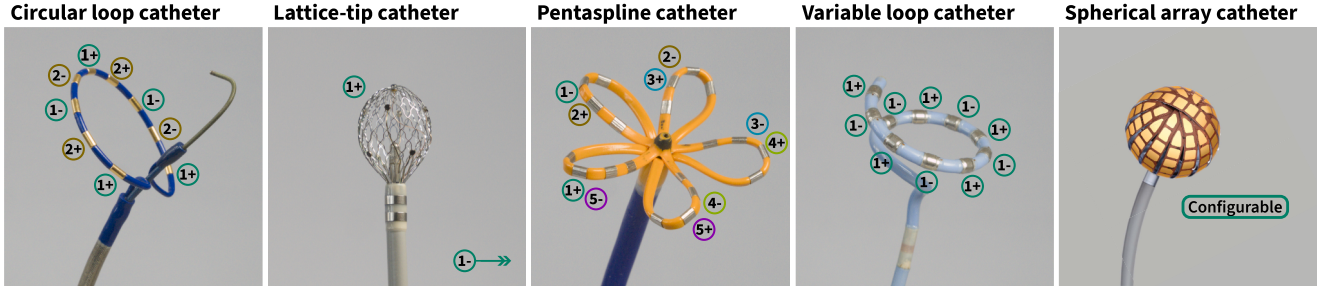
Many of the attributes of lesion size and side effects depend on the waveform and catheter design. A waveform is defined by the pulse shape, duration, pauses between the pulses, and the number of pulses. Monophasic pulses are more efficient in electroporating cells and thus creating lesion, but they also cause electrochemical reactions at the electrode-electrolyte interface, including electrolysis of water with associated gas generation, and more intensely stimulate nerves and muscles and are more painful.[43] Short biphasic pulses (which are mostly used in contemporary PFA systems) cause less neuromuscular capture[55,56] and electrochemistry[76] but also require higher amplitudes for the same level of electroporation.[77] A very important "property" of the waveform is also the duty cycle, which tells how much energy is delivered in a unit of time. The pulses need to be delivered at a sufficiently low duty cycle, that is, repetition rate, not to cause excessive heating.[39] When pulse delivery is synchronized with the R wave to avoid VT induction, the train of pulses needs to be shorter than 200 ms. Therefore, often more than 1 train of pulses is delivered.

Catheter design and waveform are most probably responsible for microembolic signals[78] and silent cerebral events and lesions.[79] In addition, the degree of hemolysis seems to differ among different catheters and waveforms.[80] However, it seems consistent across PFA systems that contact of the catheter with tissue reduces the level of hemolysis, and that level of hemolysis is correlated with the number of applications.[81]

PFA is less dependent on contact force and not critically dependent on contact.[82–84] The blood represents a conductive medium and will bridge the gap between the catheter and the cardiac tissue. Nevertheless, the lesion will be less than optimal as the effective electric field in the tissue will be lower and the depth of the lesion shallower. It has been shown that, in addition to suboptimal lesion depth, hemolysis is larger when electrodes are not in contact with the tissue.[81,85] Large multielectrode catheters come with 9–20 electrodes, and admittedly, all electrodes are virtually impossible to be in contact at the same time, meaning that at least in some places treatment is suboptimal.[86,87] 5 catheters currently approved for use by the Food and Drug Administration are presented in Figure 4. When delivered in a bipolar fashion between neighboring electrodes, the electric field in tissue will be less spread than when delivered between different splines or in a unipolar way, so different extents and intensities of all effects described earlier are expected.

Adverse events and underlying mechanisms

The absence of "traditional" complications, that is, absence of esophageal damage and most notably atrioesophageal fistula[88,89] and pulmonary vein stenosis,[90,91] has been shown in preclinical studies and has also been confirmed in clinical



Circular loop catheter	Lattice-tip catheter	Pentaspline catheter	Variable loop catheter	Spherical array catheter
Amplitude: 1500 V	**Amplitude:** <2000 V; 24-32 A	**Amplitude:** 1800-2000 V	**Amplitude:** 1800 V	**Amplitude:** 1700 V
Vectoring: Bipolar	**Vectoring:** Monopolar	**Vectoring:** Bipolar	**Vectoring:** Bipolar	**Vectoring:** Bipolar
Activation pattern: 2 half-trains with skipped electrodes	**Activation pattern:** Simple	**Activation pattern:** Between adjacent splines	**Activation pattern:** Each electrode to adjacent electrodes. Some electrodes Can be disengaged.	**Activation pattern:** Up to 64 electrodes can be engaged simultaneously.
Electrode material: Gold	**Electrode material:** Nitinol	**Electrode material:** Platinum-iridium	**Electrode material:** Platinum-iridium	**Electrode material:** Heparin coated gold
Irrigation: Non-irrigated	**Irrigation:** Irrigated	**Irrigation:** Non-irrigated	**Irrigation:** Irrigated	**Irrigation:** Non-irrigated

Figure 4
Current lineup of Food and Drug Administration–approved pulsed field ablation catheters. The figure shows the disclosed information about the different catheter implementations. Each catheter is equipped with numbers, which show how electrodes are activated in sequence. For the circular loop and pentaspline catheter, the waveform is composed of more parts. Numbers next to the electrodes show which electrodes are active in a certain part of the entire waveform.

trials and reports. The absence of these side effects is ascribed to the nonthermal nature of PFA. However, it also implies the "tissue selectivity" described earlier, which stems from the ability of most tissues to heal even after cellular death from PFA, rather than the fact that PFA cannot injure these tissues in the first place.[57,92] This healing capacity occurs because PFA spares the extracellular matrix—the scaffold essential for tissue regeneration.

Interestingly, other "energy-related" and often considered "PFA-specific" side effects such as coronary vasospasm, transient phrenic nerve palsy, SCLs, and effects on the cardiac conduction system have also been noted. None of these side effects are truly PFA specific in the sense that they would only be observed with PFA, given that they have all been described also in radiofrequency ablation (RFA) and cryoablation. In addition, hemolysis and potential arrhythmogenicity have been identified as true "PFA specific" side effects.

Coronary spasm and narrowing of the vascular lumen are a major concern and have been associated with the proximity of PFA administration to the coronaries. In the field of oncology, it is well documented that the delivery of high-voltage pulses leads to disruption of blood flow at the microcirculatory level, reducing tissue perfusion but also increasing vascular permeability.[53,58] Interestingly, electric pulses have also been studied to achieve hemostasis in noncompressible wounds,[93] and although coronary spasm was initially believed to be only an acute problem caused by PFA-mediated contraction of vascular smooth muscles that is solvable with pretreatment of high doses of nitroglycerin,[94] it was recently shown in a clinical study using optical coherence tomography that coronary spasm can be followed by neointimal hyperplasia, leading to chronic narrowing of the lumen.[95] Like any other cell type, vascular smooth muscle cells can also be damaged by high-voltage pulses.[96] In contrast, damage to smooth muscle has been associated with intimal hyperplasia.[97]

Recently, delayed, remote, and generalized coronary artery spasm occurring after isolation of pulmonary veins and posterior left atrial wall with 3 different PFA devices leading to cardiac arrest have been reported.[98–101] The incidence of this kind of coronary spasm was 0.02% in a large observational retrospective study including procedures with a pentaspline PFA catheter.[102] Importantly, this is not necessarily a "PFA specific" side effect because it was observed in the pre-PFA era. In a large Japanese retrospective registry, the reported incidence of remote coronary spasm was 0.04% and 0.34%, with RFA and cryoablation, respectively.[103] Authors have suggested that cardiac autonomic nervous system imbalance might be the causative mechanism, with cases occurring after ablation owing to injury of the epicardial parasympathetic ganglia.[103]

Regulation of coronary blood flow is dynamic, complex, and not well understood.[104] Electric pulses may, in addition to causing damage to vascular smooth muscles or their contraction like in proximity-related vasospasm, stimulate (or stun) sympathetic and parasympathetic innervation and thus cause autonomic nervous system imbalance. Furthermore, electroporation was shown to increase vascular permeability and cause edema,[52,53] thus increasing microvascular resistance that is also involved in the regulation of coronary blood flow. Other mechanisms, such as the vasoconstrictive effects of free heme and oxyhemoglobin after intravascular PFA-mediated hemolysis, might also have a role.[98] Further research to understand and possibly discover preventive measures for this very rare but dangerous adverse effect is necessary.

Experience with thermal ablation procedures has shown that phrenic nerve injury (PNI) is possible when structures close to its course are ablated, that is, the anterior part of the right superior pulmonary vein or the ostium of the superior vena cava. PNI is mainly related to cryoablation, and most cases are transient or show delayed recovery, with the incidence of persistent PNI estimated at 0.2%–0.3%.[105,106] Preclinical data suggest that PFA can cause temporary effects on phrenic nerve function, such as stunning, which likely results from depolarization of the nerve axonal membranes owing to the nonselective leak current associated with electroporation (Figure 2B).[107] Importantly, the effects seem to be transient, with nerve function recovering within the procedure and no histologic changes indicating structural tissue damage.[108–110]

The results of most clinical studies and registries are consistent with preclinical data, showing only transient PNI and no long-term palsy.[9,111,112] Recently, an analysis of PNI after a PFA procedure using the pentaspline catheter revealed a potentially high incidence of PNI that persisted for at least 3 months postprocedurally (1/5 of the 64 who underwent PFA, totaling approximately 1.5%).[92,102] The sensitivity for PNI detection was probably higher in this study than in most previous studies, given that PNI assessment based on functional fluoroscopy was used. In contrast, the short follow-up (3 months versus longer periods in most studies) with insufficient time for phrenic nerve regeneration could be the reason for this discrepancy.

The cerebral embolic complications of catheter ablation range from cerebrovascular infarctions and TIAs to SCLs. The incidence of cerebrovascular infarction and TIA when using PFA ranges from 0.3% to 1.2%, depending on the type of device used.[113–116] It seems that embolic complications are not energy specific. A recent meta-analysis has shown that the incidence of SCL was not significantly different between the pentaspline PFA catheter (14.4%) and several other catheter designs using different thermal ablation methods.[117] The high incidence of SCL (8.9%–12% in the first large PFA studies)[7,113,118] is worrying, given that it can lead to cognitive decline.[119] Recent reports of a high incidence of SCL (85.7%) with the use of a specific device[120] (albeit using a 3T scanner) suggest that catheter and waveform design may play a critical role and can be improved to reduce embolic events in the future, particularly those related to overheating during PFA delivery.[39,40]

It is currently assumed that hemolysis is a direct consequence of the electroporation of erythrocytes and often occurs after PFA for the treatment of AF.[121] Two smaller

prospective studies observed severe hemolysis leading to acute kidney injury (AKI) in 2.8% and 2.9%.[122] However, the incidence of AKI owing to severe hemolysis was low in a large retrospective observational study (0.03%) and related to a very high number of PFA applications with a pentaspline catheter (143 per procedure).[9,102] The extent of hemolysis varies among systems and is best correlated with the size of the electrodes, electric field intensity used,[123] the number of applications, and the catheter design.[80,124] In addition, it depends on the volume of the blood that can be exposed to intense electric fields. This volume is smaller with balloon-type catheters owing to the occlusion of the blood flow[125] and greater with catheters with large surface area or number of electrodes; in the case of bad contact of the catheter with the myocardium, it becomes even higher.[85] Improving electrode contact and limiting the number of applications were suggested to minimize hemolysis,[81,121] and hydration after ablation was suggested to prevent AKI,[126] although that does not always guarantee avoidance of kidney injury.[127]

There is some evidence that electroporation affects the cardiac conductive system (CCS) in a different manner than the working myocardium. An ex vivo study on perfused canine hearts demonstrated different susceptibility of various parts of the CCS to electroporation.[128] In their experiments, authors have shown higher resistance of the left bundle branch and the His area to electroporation, which might be attributed to the fibrous sheaths covering the bundle of His and the proximal part of the left bundle, thus shielding those structures from the electric field.[129] A similar finding with histologic evidence of His bundle viability with evident surrounding myocardial damage after electroporation was revealed in a preclinical study on dogs.[130] Indeed, there are some reports of recovery of the proximal CCS function after intentional or unintentional electroporation and acute conduction block.[131,132] Histologic analyses from animal studies show that the distal parts of the CCS, the Purkinje fibers, may also be less sensitive to electroporation; however, the proposed mechanisms are less clear.[133–135] The evidence regarding the effect of electroporation on the CCS is still emerging, and although sparing has been observed, it is not yet fully understood, if this is consistent across different experimental and clinical scenarios. Therefore, careful consideration is warranted when delivering PFA in proximity to the CCS.

Periprocedural and postprocedural markers

All currently approved and most existing systems have been developed (or adapted) for PVI and thus for ablation of a thin atrial wall of 2–5 mm. This seems to be easily achievable, given that all systems work quite well with careful and conscientious use, that is, careful positioning and repositioning of the catheter. The real-world data look different—early PFA results show reconnections at redo procedures, and even acute PVI and linear lines are not always easy to achieve.[8] Even LGE cardiac MRI 3 months after index procedure shows gaps in 20% of patients[75] and incomparably worse images than after RFA and cryoablation.[74] This indicates the need to develop and validate new and more precise periprocedural markers to achieve consistently good results. Lesion formation shows interesting dynamics on LGE MRI and gross pathology,[52,69,84] suggesting that PFA lesion development has a different time course than thermal modalities, as visualized by medical imaging. Especially prominent is the relatively large reduction in size of hyperintense regions between 1 and 7 days after treatment.

Although the disappearance of iEGM signals was very impressive in early preclinical studies, it was quickly realized that the electrograms can gradually return after treatment.[33,136] However, there seems to be some correlation with the rate of recovery and lesion size on MRI and gross pathology.[52] The possibility of using less intense (sublethal) PFA deliveries to cause a transient disappearance of iEGMs through the basic electroporation mechanisms described earlier offers the possibility to use these sublethal applications to identify isthmus for arrhythmia reentry. This has been recently reported[137]; however, it was not always possible to eliminate the identified isthmus with PFA, and investigators resorted to complete ablations using radiofrequency. This clearly shows the need to determine and identify the difference between stunning by reversible electroporation and achieving cell death by irreversible electroporation.

PFA in the ventricles seems like a logical next step. However, current systems, which were developed for ablation in the atria where tissue is rather thin, have been demonstrated to achieve a maximum 6–8 mm lesion depth on average in preclinical studies, which is not sufficient for achieving transmural/deep lesions in the left ventricles.[69,138] PFA in ventricles will also most probably require high-voltage pulses to be synchronized with the R wave in spite of the lack of arrhythmogenicity demonstrated with biphasic short pulses in preclinical studies.[139,140] Importantly, stunning beyond the ablation dimensions can potentially cause safety concerns. Given that recovery can sometimes require up to 30 minutes, this can potentially result in acute worsening of the ventricular function. However, the same effect can also lead to transient elimination of arrhythmia inducibility, without achieving a permanent effect. This means that VT noninducibility after PFA treatment is not necessarily a reliable marker for long-term outcomes.

Combination with other ablation technologies/energies

Some PFA systems were initially developed as pure RFA systems and only later, during the design phase, modified to include PFA ablation. These systems now feature the possibility to combine RFA and PFA ablation. This also enables combination therapies to be evaluated including a combination of RFA and PFA[141] and PFA and cryoablation.[142] The rationale for why different combinations would work better than each energy alone is most often superficial and incomplete, and evidence is also scarce. In other areas of

electroporation use, it has been shown that mild heating combined with electroporation increases electroporation efficiency in bacterial inactivation,[143] increases lesion size[144,145] and gene transfection efficiency.[146] Mild radiofrequency heating has also shown an increase in electroporation efficiency in vitro.[147,148] The combined treatments will likely result in prolonged treatment times and potentially bring back adverse events of thermal ablation energies, thus losing the exact benefits of PFA.

Conclusion

Despite the rapid clinical adoption and promising safety profile of PFA, important questions remain about the dynamics and complex myocardial response after pulse treatment. In particular, the interplay between reversible and irreversible electroporation pathways and their contribution to lesion durability and tissue recovery is not yet fully understood. A deeper mechanistic insight into these processes is essential to optimize lesion formation and minimize unintended effects and adverse events. In addition, proximity sensing and better integration of PFA with advanced navigation and imaging systems will likely represent the next significant advance, enabling improved procedural control, real-time feedback, and personalization of ablation strategies. As experience with the current generation of PFA devices grows, so does the understanding of their capabilities and limitations. It is anticipated that the next development cycle of PFA systems will build on this growing body of knowledge by incorporating insights on the mechanisms described to further improve safety, efficacy, and long-term outcomes in the treatment of atrial and ventricular arrhythmias.

Patents

TIMING OF PULSED FIELD ABLATION ENERGY DELIVERIES (publication number: 20240016540; type: application; filed: June 20, 2023; publication date: January 18, 2024; inventors: Daniel C. Sigg, Lars M. Mattison, and Damijan Miklavčič), MULTIPLE PULSE WIDTH TRAINS TO ENHANCE ABLATION HOMOGENEITY IN HIGHLY ORIENTED CELLULAR SUBSTRATES (publication number: 20230310061; type: application; filed: April 4, 2023; publication date: October 5, 2023; inventors: Mark T. Stewart, Lars M. Mattison, Brian T. Howard, Damijan Miklavčič, Tadej Kotnik, Janja Dermol-Cerne, and Tina Batista Napotnik), PULSE WAVE OPTIMIZATION TO MINIMIZE NEUROMUSCULAR STIMULATION DURING PULSED FIELD ABLATION TREATMENT (inventors: Daniel C. Sigg, Damijan Miklavčič, Lars Mattison, Aleksanda Cvetkoska, Alenka Macek Lebar, and Matej Rebersek; MBF ref: 215364-0041-US02; MDT ref: A0010673US02).

Funding Sources: The work was in part supported by the Slovenian Research and Innovation Agency (ARIS) through P2-0249 and I0-0022. The study was in part supported by ARIS and the University of Ljubljana through funding for Start-Up Research Programmes. The study was in part supported by the European Union and ARIS through NextGenerationEU and NOO funding within project MN-0023. The study was in part supported by the European Union's Horizon Europe research and innovation program within the ERC Starting Grant project no. 101115323—REINCARNATION. Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or the European Research Council. Neither the European Union nor the granting authority can be held responsible for them.

Disclosures: D. Miklavčič: consulting for Medtronic and Inomagen; honoraria and travel support from Medtronic, Field Medical, and Boston Scientific. B. Kos: consulting for Medtronic. M. Jan: proctorship fees from Johnson & Johnson and Abbot; honoraria from Medtronic.

Address reprint requests and correspondence: Dr Damijan Miklavčič, University of Ljubljana, Faculty of Electrical Engineering, Ljubljana, Slovenia. E-mail address: damijan.miklavcic@fe.uni-lj.si

References

1. Chun KJ, Miklavčič D, Vlachos K, et al. State-of-the-art pulsed field ablation for cardiac arrhythmias: ongoing evolution and future perspective. Europace 2024;26:euae134.
2. Maizels L, Kalman JM. Pulsed-field ablation: a revolution in atrial fibrillation therapy. Nat Rev Cardiol 2024;21:519–520.
3. Di Biase L, Merino J, Natale A. Pulsed field ablation for ventricular tachycardia: are we there yet? Europace 2025;27:euaf161.
4. Sacher F, Sarkozy A, Pürerfellner H, et al. Safety and efficacy of a lattice-tip catheter for ventricular arrhythmia ablation: the AFFERA Ventricular Arrhythmia Ablation Registry (AVAAR). Europace 2025;27:euaf139.
5. Zhang Z, Xiao Y, Wang C, et al. Pulsed field ablation: a promising approach for ventricular tachycardia ablation. Int J Cardiol 2024;407:131985.
6. Reichlin T, Kueffer T, Badertscher P, et al. Pulsed field or cryoballoon ablation for paroxysmal atrial fibrillation. N Engl J Med 2025;392:1497–1507.
7. Reddy VY, Gerstenfeld EP, Natale A, et al. Pulsed field or conventional thermal ablation for paroxysmal atrial fibrillation. N Engl J Med 2023;389:1660–1671.
8. Scherr D, Turagam MK, Maury P, et al. Repeat procedures after pulsed field ablation for atrial fibrillation: MANIFEST-REDO study. Europace 2025;27:euaf012.
9. Ekanem E, Neuzil P, Reichlin T, et al. Safety of pulsed field ablation in more than 17,000 patients with atrial fibrillation in the MANIFEST-17K study. Nat Med 2024;30:2020–2029.
10. Boersma LVA, Széplaki G, Dello Russo A, et al. Real-world experience with the pentaspline pulsed field ablation system: one-year outcomes of the FARADISE registry. Europace 2025;27:euaf182.
11. Mahnič-Kalamiza S, Vorobiev E, Miklavčič D. Electroporation in food processing and biorefinery. J Membr Biol 2014;247:1279–1304.
12. Geboers B, Scheffer HJ, Graybill PM, et al. High-voltage electrical pulses in oncology: irreversible electroporation, electrochemotherapy, gene electrotransfer, electrofusion, and electroimmunotherapy. Radiology 2020;295:254–272.
13. Kotnik T, Frey W, Sack M, Haberl Meglič S, Peterka M, Miklavčič D. Electroporation-based applications in biotechnology. Trends Biotechnol 2015;33:480–488.
14. Kotnik T, Rems L, Tarek M, Miklavčič D. Membrane electroporation and electropermeabilization: mechanisms and models. Annu Rev Biophys 2019;48:63–91.
15. Kotnik T, Pucihar G, Miklavcic D. Induced transmembrane voltage and its correlation with electroporation-mediated molecular transport. J Membr Biol 2010;236:3–13.
16. Sengel JT, Wallace MI. Imaging the dynamics of individual electropores. Proc Natl Acad Sci U S A 2016;113:5281–5286.
17. Rems L, Kasimova MA, Testa I, Delemotte L. Pulsed electric fields can create pores in the voltage sensors of voltage-gated ion channels. Biophys J 2020;119:190–205.
18. Wiczew D, Szulc N, Tarek M. Molecular dynamics simulations of the effects of lipid oxidation on the permeability of cell membranes. Bioelectrochemistry 2021;141:107869.
19. Silkunas M, Silkuniene G, Pakhomov AG. Real-time imaging of individual electropores proves their longevity in cells. Biochem Biophys Res Commun 2024;695:149408.
20. Rols MP, Teissié J. Electropermeabilization of mammalian cells. Quantitative analysis of the phenomenon. Biophys J 1990;58:1089–1098.
21. Polajzer T, Jarm T, Miklavcic D. Analysis of damage-associated molecular pattern molecules due to electroporation of cells in vitro. Radiol Oncol 2020;54:317–328.

22. Blažič A, Guinard M, Leskovar T, O'Connor RP, Rems L. Long-term changes in transmembrane voltage after electroporation are governed by the interplay between nonselective leak current and ion channel activation. Bioelectrochemistry 2025;161:108802.
23. Graybill PM, Davalos RV. Cytoskeletal disruption after electroporation and its significance to pulsed electric field therapies. Cancers (Basel) 2020;12:1132.
24. Polajžer T, Peng W, Yao C, Miklavčič D. Changes in mitochondrial membrane potential in *in vitro* electroporation with nano- and microsecond pulses. Bioelectricity 2024;6:97–107.
25. Batista Napotnik T, Polajžer T, Miklavčič D. Cell death due to electroporation – a review. Bioelectrochemistry 2021;141:107871.
26. Arciga BM, Walters DM, Kimchi ET, et al. Pulsed electric field ablation as a candidate to enhance the anti-tumor immune response to immune checkpoint inhibitors. Cancer Lett 2025;609:217361.
27. Peng W, Polajžer T, Yao C, Miklavčič D. Dynamics of cell death due to electroporation using different pulse parameters as revealed by different viability assays. Ann Biomed Eng 2024;52:22–35.
28. Chaigne S, Sigg DC, Stewart MT, et al. Reversible and irreversible effects of electroporation on contractility and calcium homeostasis in isolated cardiac ventricular myocytes. Circ Arrhythm Electrophysiol 2022;15:e011131.
29. Jones JL, Lepeschkin E, Jones RE, Rush S. Response of cultured myocardial cells to countershock-type electric field stimulation. Am J Physiol 1978; 235:H214–H222.
30. Tung L, Tovar O, Neunlist M, Jain SK, O'Neill RJ. Effects of strong electrical shock on cardiac muscle Tissuea. Ann N Y Acad Sci 1994;720:160–175.
31. Wegner LH, Frey W, Silve A. Electroporation of DC-3F cells is a dual process. Biophys J 2015;108:1660–1671.
32. Batista Napotnik T, Kos B, Jarm T, Miklavčič D, O'Connor RP, Rems L. Genetically engineered HEK cells as a valuable tool for studying electroporation in excitable cells. Sci Rep 2024;14:720.
33. Štublar J, Jarm T, Mattison L, et al. Intracardiac electrogram analysis may allow for prediction of lesion transmurality after pulsed field ablation of atria in a porcine model. Heart Rhythm O2 2024;6:350–361.
34. Steuer A, Zhuang J, Kolb JF, Daeschlein G. Effect of pulsed electric fields on cell-cell-communication via gap junctions in cell-monolayers Paper presented at. San Francisco, CA, USA: 2013 Abstracts IEEE International Conference on Plasma Science (ICOPS); 2013. 1–1.
35. Hunter DW, Kostecki G, Fish JM, Jensen JA, Tandri H. In vitro cell selectivity of reversible and irreversible: electroporation in cardiac tissue. Circ Arrhythm Electrophysiol 2021;14:e008817.
36. Jan V, Jusović M, Miklavčič D. Effects of electroporation on the function of Sarco/endoplasmic reticulum Ca^{2+}-ATPase and Na^+, K^+-ATPase in H9c2 cells. Appl Sci 2024;14:2695.
37. Dermol-Černe J, Batista Napotnik T, Reberšek M, Miklavčič D. Short microsecond pulses achieve homogeneous electroporation of elongated biological cells irrespective of their orientation in electric field. Sci Rep 2020;10:9149.
38. Scuderi M, Dermol-Černe J, Batista Napotnik T, et al. Characterization of experimentally observed complex interplay between pulse duration, electrical field strength, and cell orientation on electroporation outcome using a time-dependent nonlinear numerical model. Biomolecules 2023;13:727.
39. Mahnič-Kalamiza S, Miklavčič D, Lombergar P, et al. Elucidating the mechanisms of microbubble formation in intracardiac pulsed field ablation. Electrochim Acta 2024;497:144550.
40. Koop B. Fundamentals of system design for cardiac pulsed field ablation: optimization of safety, efficacy, and usability. Pacing Clin Electrophysiol 2025. Epub ahead of print.
41. Kos B, Reberšek M, Miklavčič D. Complexity and perplexity of pulsed field ablation: an engineering perspective. Arrhythm Electrophysiol Rev 2025;14:e24.
42. Kos B, Mattison L, Ramirez D, et al. Determination of lethal electric field threshold for pulsed field ablation in *ex vivo* perfused porcine and human hearts. Front Cardiovasc Med 2023;10:1160231.
43. Mercadal B, Arena CB, Davalos RV, Ivorra A. Avoiding nerve stimulation in irreversible electroporation: a numerical modeling study. Phys Med Biol 2017; 62:8060–8079.
44. Casciola M, Xiao S, Pakhomov AG. Damage-free peripheral nerve stimulation by 12-ns pulsed electric field. Sci Rep 2017;7:10453.
45. Tan NY, Del-Carpio Munoz F, Tri JA, et al. Atrial fibrillation and flutter conversion with pulsed electric field delivery: preclinical proof of concept. J Interv Card Electrophysiol 2025. Epub ahead of print.
46. Cukjati D, Batiuskaite D, André F, Miklavcic D, Mir LM. Real time electroporation control for accurate and safe in vivo non-viral gene therapy. Bioelectrochemistry 2007;70:501–507.
47. Ivorra A, Al-Sakere B, Rubinsky B, Mir LM. In vivo electrical conductivity measurements during and after tumor electroporation: conductivity changes reflect the treatment outcome. Phys Med Biol 2009;54:5949–5963.
48. Bhonsle S, Lorenzo MF, Safaai-Jazi A, Davalos RV. Characterization of nonlinearity and dispersion in tissue impedance during high-frequency electroporation. IEEE Trans Biomed Eng 2018;65:2190–2201.
49. Jacobs Iv EJ, Campelo SN, Charlton A, Altreuter S, Davalos RV. Characterizing reversible, irreversible, and calcium electroporation to generate a burst-dependent dynamic conductivity curve. Bioelectrochemistry 2024; 155:108580.
50. Pavlin M, Miklavcic D. The effective conductivity and the induced transmembrane potential in dense cell system exposed to DC and AC electric fields. IEEE Trans Plasma Sci 2009;37:99–106.
51. Duck FA. Physical Properties of Tissue: A Comprehensive Reference Book. New York, NY: Academic Press; 1990.
52. Miklavčič D, Verma A, Krahn PRP, et al. Biophysics and electrophysiology of pulsed field ablation in normal and infarcted porcine cardiac ventricular tissue. Sci Rep 2024;14:32063.
53. Markelc B, Bellard E, Sersa G, et al. Increased permeability of blood vessels after reversible electroporation is facilitated by alterations in endothelial cell-to-cell junctions. J Control Release 2018;276:30–41.
54. Steendijk P, van der Velde ET, Baan J. Dependence of anisotropic myocardial electrical resistivity on cardiac phase and excitation frequency. Basic Res Cardiol 1994;89:411–426.
55. Cvetkoska A, Maček-Lebar A, Trdina P, Miklavčič D, Reberšek M. Muscle contractions and pain sensation accompanying high-frequency electroporation pulses. Sci Rep 2022;12:8019.
56. Cvetkoska A, Maček-Lebar A, Polajžer T, et al. The effects of interphase and interpulse delays and pulse widths on induced muscle contractions, pain and therapeutic efficacy in electroporation-based therapies. J Cardiovasc Dev Dis 2023;10:490.
57. Nies M, Koruth JS, Mlček M, et al. Is the esophagus spared during pulsed field ablation? Early histopathology and in vivo esophageal retraction. Heart Rhythm 2025. Epub ahead of print.
58. Jarm T, Cemazar M, Miklavcic D, Sersa G. Antivascular effects of electrochemotherapy: implications in treatment of bleeding metastases. Expert Rev Anticancer Ther 2010;10:729–746.
59. Kaminska I, Kotulska M, Stecka A, et al. Electroporation-induced changes in normal immature rat myoblasts (H9C2). Gen Physiol Biophys 2012;31:19–25.
60. Casciola M, Keck D, Feaster TK, Blinova K. Human cardiomyocytes are more susceptible to irreversible electroporation by pulsed electric field than human esophageal cells. Physiol Rep 2022;10:e15493.
61. Vogel JA, van Veldhuisen E, Agnass P, et al. Time-dependent impact of irreversible electroporation on pancreas, liver, blood vessels and nerves: a systematic review of experimental studies. PLoS One 2016;11:e0166987.
62. Kinosita K Jr, Tsong TY. Formation and resealing of pores of controlled sizes in human erythrocyte membrane. Nature 1977;268:438–441.
63. Maček-Lebar A, Miklavčič D. Cell electropermeabilization to small molecules in vitro: control by pulse parameters. Radiol Oncol 2001;35:193–202.
64. Pucihar G, Krmelj J, Reberšek M, Napotnik TB, Miklavčič D. Equivalent pulse parameters for electroporation. IEEE Trans Biomed Eng 2011;58:3279–3288.
65. Dermol J, Miklavčič D. Mathematical models describing Chinese hamster ovary cell death due to electroporation in vitro. J Membr Biol 2015;248:865–881.
66. Watanabe K, Nies M, Reddy VY, Koruth JS. Lesion morphometry of the pentaspline pulsed field ablation catheter: understanding catheter pose, rotation, and dosing. Circ Arrhythm Electrophysiol 2024;17:e013208.
67. Yavin HD, Higuchi K, Sroubek J, Younis A, Zilberman I, Anter E. Pulsed-field ablation in ventricular myocardium using a focal catheter: the impact of application repetition on lesion dimensions. Circ Arrhythm Electrophysiol 2021; 14:e010375.
68. Verma A, Asivatham SJ, Deneke T, Castellvi Q, Neal RE 2nd. Primer on pulsed electrical field ablation: understanding the benefits and limitations. Circ Arrhythm Electrophysiol 2021;14:e010086.
69. Kueffer T, Casoni D, Goepfert C, et al. Dose-dependent ventricular lesion formation using a novel large-area pulsed field ablation catheter: a preclinical feasibility study. Heart Rhythm 2025;22:2322–2330.
70. Petras A, Amoros Figueras G, Moreno Weidmann Z, et al. Is a single lethal electric field threshold sufficient to characterize the lesion size in computational modeling of cardiac pulsed-field ablation? Heart Rhythm O2 2025;6:671–677.
71. Castellvi Q, Ivorra A. Computational multiscale modeling of pulsed field ablation considering conductivity and damage anisotropy reveals deep lesion morphologies. Int J Numer Methods Biomed Eng 2025;41:e70077.
72. Grimaldi M, Di Monaco A, Gomez T, et al. Time course of irreversible electroporation lesion development through short- and long-term follow-up in pulsed-field ablation–treated hearts. Circ Arrhythm Electrophysiol 2022; 15:e010661.
73. Barabasch A, Distelmaier M, Heil P, Krämer NA, Kuhl CK, Bruners P. Magnetic resonance imaging findings after percutaneous irreversible electroporation of liver metastases: a systematic longitudinal study. Invest Radiol 2017;52:23–29.
74. Hermans BJM, Farnir FIP, Bijvoet GP, et al. Visualization of monopolar biphasic focal pulsed field ablation lesions 3 months after pulmonary vein isolation on high-resolution 3-dimensional dark-blood late gadolinium enhancement cardiac magnetic resonance images. Heart Rhythm 2025. Epub ahead of print.
75. Fink T, Sciacca V, Bannmann K, et al. Magnetic resonance imaging analysis of left atrial scar formation following atrial fibrillation ablation using a novel variable loop catheter for pulsed field ablation. Heart Rhythm 2025;22:e678–e679.
76. Mahnič-Kalamiza S, Miklavčič D. Scratching the electrode surface: insights into a high-voltage pulsed-field application from *in vitro & in silico* studies in indifferent fluid. Electrochim Acta 2020;363:137187.
77. Mercadal B, Beitel-White N, Aycock KN, Castellví Q, Davalos RV, Ivorra A. Dynamics of cell death after conventional IRE and H-FIRE treatments. Ann Biomed Eng 2020;48:1451–1462.
78. Shiomi S, Tokuda M, Takato U, et al. Real-time detection of microembolic signals using carotid echocardiography: a comparative study of 3 pulsed-field systems. Heart Rhythm 2025. Epub ahead of print.
79. Sánchez-Gómez JM, Ortiz-Fernández Á, Soler-Martínez J, et al. Incidence of brain lesions following pulsed field ablation for atrial fibrillation. Heart Rhythm 2025. Epub ahead of print.

80. Kawamura I, Miyazaki S, Kato R, et al. Comparison of hemolysis with different pulsed field ablation systems. Heart Rhythm 2025. Epub ahead of print.
81. Nies M, Koruth JS, Mlček M, et al. Hemolysis after pulsed field ablation: impact of lesion number and catheter-tissue contact. Circ Arrhythm Electrophysiol 2024;17:e012765.
82. Howard B, Verma A, Tzou WS, et al. Effects of electrode-tissue proximity on cardiac lesion formation using pulsed field ablation. Circ Arrhythm Electrophysiol 2022;15:e011110.
83. Mattison L, Verma A, Tarakji KG, et al. Effect of contact force on pulsed field ablation lesions in porcine cardiac tissue. J Cardiovasc Electrophysiol 2023; 34:693–699.
84. Di Biase L, Marazzato J, Govari A, et al. Pulsed field ablation index–guided ablation for lesion formation: impact of contact force and number of applications in the ventricular model. Circ Arrhythm Electrophysiol 2024;17:e012717.
85. Mattison L, Verma A, Tarakji KG, Sigg DC. Hemolysis after pulsed field ablation: the role of dose and contact in an acute porcine model. Circ Arrhythm Electrophysiol 2024;17:e013317.
86. Saito Y, Nagashima K, Watanabe R, et al. Clinical importance of tissue proximity indication during pulsed field ablation for atrial fibrillation: insights from initial experience. Heart Rhythm 2025;22:e51–e59.
87. Mills MT, Sommer P, Day J, et al. Characterization of acute residual pulmonary vein connections using electroanatomic mapping during pulsed-field ablation of atrial fibrillation. Heart Rhythm 2025;22:3140–3148.
88. Cochet H, Nakatani Y, Sridi-Cheniti S, et al. Pulsed field ablation selectively spares the oesophagus during pulmonary vein isolation for atrial fibrillation. Europace 2021;23:1391–1399.
89. Gunawardene MA, Middeldorp M, Pape UF, et al. Esophageal endoscopic findings after pulmonary vein and posterior wall isolation using pulsed field ablation: results from the Eso-PFA study. Europace 2025;27:euaf133.
90. Howard B, Haines DE, Verma A, et al. Reduction in pulmonary vein stenosis and collateral damage with pulsed field ablation compared with radiofrequency ablation in a canine model. Circ Arrhythm Electrophysiol 2020;13:e008337.
91. Mansour M, Gerstenfeld EP, Patel C, et al. Pulmonary vein narrowing after pulsed field versus thermal ablation. Europace 2024;26:euae038.
92. Chéhirlian L, Koutbi L, Mancini J, et al. High incidence of phrenic nerve injury in patients undergoing pulsed field ablation for atrial fibrillation. Heart Rhythm 2025;22:e1206–e1213.
93. Brinton M, Mandel Y, Schachar I, Palanker D. Mechanisms of electrical vasoconstriction. J Neuroeng Rehabil 2018;15:43.
94. Malyshev Y, Neuzil P, Petru J, et al. Nitroglycerin to ameliorate coronary artery spasm during focal pulsed-field ablation for atrial fibrillation. JACC Clin Electrophysiol 2024;10:885–896.
95. Tam MTK, Chan JYS, Chan CP, et al. Effect of pulsed-field ablation on human coronary arteries: a longitudinal study with intracoronary imaging. JACC Clin Electrophysiol 2025;11:1478–1488.
96. Maor E, Ivorra A, Leor J, Rubinsky B. The effect of irreversible electroporation on blood vessels. Technol Cancer Res Treat 2007;6:307–312.
97. Déglise S, Bechelli C, Allagnat F. Vascular smooth muscle cells in intimal hyperplasia, an update. Front Physiol 2023;13:1081881.
98. Luther V, Chiong J, James C, Modi S, Gupta D, Hung J. Diffuse right coronary artery spasm occurring 45 minutes after pulsed field ablation for atrial fibrillation. Heart Rhythm 2025;22:1864–1867.
99. Mannion J, Gorry C, Foley B, Tuohy S. Remote diffuse multi-coronary artery spasm following pulmonary vein and posterior wall isolation, using a focal monopolar pulsed field ablation catheter. HeartRhythm Case Rep 2025; 11:1113–1119.
100. Matsuura G, Fukaya H, Yoshizawa T, Sugimoto A, Ako J. Delayed coronary artery vasospasm leading to cardiac arrest after pulsed field ablation for atrial fibrillation. Heart Rhythm 2025. Epub ahead of print.
101. Hayasaka K, Peichl P, Ventrella N, et al. Coronary vasospasm during pulsed field focal ventricular ablation with solid tip catheter. Europace 2025; 27:euaf226.
102. Turagam MK, Aryana A, Day JD, et al. Multicenter study on the safety of pulsed field ablation in over 40,000 patients: MANIFEST-US. J Am Coll Cardiol 2025. Epub ahead of print.
103. Nakamura T, Takami M, Fukuzawa K, et al. Incidence and characteristics of coronary artery spasms related to atrial fibrillation ablation procedures large-scale multicenter analysis. Circ J 2021;85:264–271.
104. Sato T, Hanna P, Mori S. Innervation of the coronary arteries and its role in controlling microvascular resistance. J Cardiol 2024;84:1–13.
105. Sacher F, Jais P, Stephenson K, et al. Phrenic nerve injury after catheter ablation of atrial fibrillation. Indian Pacing Electrophysiol J 2007;7:1–6.
106. Kuck KH, Brugada J, Fürnkranz A, et al. Cryoballoon or radiofrequency ablation for paroxysmal atrial fibrillation. N Engl J Med 2016;374:2235–2245.
107. Rems L, Rainot A, Wiczew D, Szulc N, Tarek M. Cellular excitability and ns-pulsed electric fields: potential involvement of lipid oxidation in the action potential activation. Bioelectrochemistry 2024;155:108588.
108. Howard B, Haines DE, Verma A, et al. Characterization of phrenic nerve response to pulsed field ablation. Circ Arrhythm Electrophysiol 2022; 15:e010127.
109. Hsu JC, Gibson D, Banker R, et al. In vivo porcine characterization of atrial lesion safety and efficacy utilizing a circular pulsed-field ablation catheter including assessment of collateral damage to adjacent tissue in supratherapeutic ablation applications. J Cardiovasc Electrophysiol 2022;33:1480–1488.
110. Yavin H, Shapira-Daniels A, Barkagan M, et al. Pulsed field ablation using a lattice electrode for focal energy delivery: biophysical characterization, lesion durability, and safety evaluation. Circ Arrhythm Electrophysiol 2020; 13:e008580.
111. Metzner A, Fiala M, Vijgen J, et al. Long-term outcomes of the pentaspline pulsed-field ablation catheter for the treatment of paroxysmal atrial fibrillation: results of the prospective, multicentre FARA-Freedom Study. Europace 2024; 26:euae053.
112. Ollitrault P, Chaumont C, Font J, et al. Superior vena cava isolation using a pentaspline pulsed-field ablation catheter: feasibility and safety in patients undergoing atrial fibrillation catheter ablation. Europace 2024;26:euae160.
113. Verma A, Haines DE, Boersma LV, et al. Pulsed field ablation for the treatment of atrial fibrillation: PULSED AF pivotal trial. Circulation 2023; 147:1422–1432.
114. Turagam MK, Neuzil P, Schmidt B, et al. Safety and effectiveness of pulsed field ablation to treat atrial fibrillation: one-year outcomes from the MANIFEST-PF registry. Circulation 2023;148:35–46.
115. Reddy VY, Calkins H, Mansour M, et al. Pulsed field ablation to treat paroxysmal atrial fibrillation: safety and effectiveness in the AdmIRE pivotal trial. Circulation 2024;150:1174–1186.
116. Dar BY, Yusuf YA, Esmati MH. Analysis of pulsed field ablation using focal contact force-sensing catheters for treatment of atrial fibrillation: acute and 90-day invasive remapping results. Europace 2023;25:euad287.
117. Hu X, Li W, Ren B, Zeng R. Incidence of silent cerebral events detected by MRI in patients with atrial fibrillation undergoing pulsed field ablation vs thermal ablation: a systematic review and network meta-analysis. Heart Rhythm 2025. Epub ahead of print.
118. Duytschaever M, De Potter T, Grimaldi M, et al. Paroxysmal atrial fibrillation ablation using a novel variable-loop biphasic pulsed field ablation catheter integrated with a 3-dimensional mapping system: 1-year outcomes of the multicenter inspIRE study. Circ Arrhythm Electrophysiol 2023;16:e011780.
119. Gaita F, Corsinovi L, Anselmino M, et al. Prevalence of silent cerebral ischemia in paroxysmal and persistent atrial fibrillation and correlation with cognitive function. J Am Coll Cardiol 2013;62:1990–1997.
120. Miyazaki S, Kawamura I, Iwasa Y, et al. Different incidence and size of silent strokes after pulsed field ablation with circular shaped ablation catheters. Circ Arrhythm Electrophysiol 2025;18:e013719.
121. Popa MA, Venier S, Menè R, et al. Characterization and clinical significance of hemolysis after pulsed field ablation for atrial fibrillation: results of a multicenter analysis. Circ Arrhythm Electrophysiol 2024;17:e012732.
122. Venier S, Vaxelaire N, Jacon P, et al. Severe acute kidney injury related to haemolysis after pulsed field ablation for atrial fibrillation. Europace 2023; 26:euad371.
123. Fiserova I, Fiser O, Novak M, et al. Significant hemolysis is present during irreversible electroporation of cardiomyocytes in vitro. Heart Rhythm 2025; 22:466–474.
124. Shinohara M, Oates C, Power JR, et al. A comparative analysis of hemolysis related to the bipolar pentaspline and monopolar lattice-tip catheters. JACC Clin Electrophysiol 2025. Epub ahead of print.
125. Lo M, Gambhir A, Sundaram S, et al. Safety and effectiveness of a novel balloon-in-basket pulsed-field ablation catheter for the treatment of paroxysmal and persistent AF: volt-AF IDE trial acute results. Heart Rhythm 2025; 22:2524–2533.
126. Mohanty S, Casella M, Compagnucci P, et al. Acute kidney injury resulting from hemoglobinuria after pulsed-field ablation in atrial fibrillation: is it preventable? JACC Clin Electrophysiol 2024;10:709–715.
127. Boyle TA, Frankel DS. Severe acute kidney injury from limited pulsed field ablation. Heart Rhythm 2025;22:e1043.
128. Livia C, Sugrue A, Witt T, et al. Elimination of Purkinje fibers by electroporation reduces ventricular fibrillation vulnerability. J Am Heart Assoc 2018;7:e009070.
129. Sánchez-Quintana D, Yen Ho S. Anatomía de los nodos cardíacos y del sistema de conducción específico auriculoventricular [Anatomy of cardiac nodes and atrioventricular specialized conduction system]. Rev Esp Cardiol 2003; 56:1085–1092.
130. Zhai Z, Wang Y, Shi L, Liu X. Impact of pulsed electric field ablation on His bundle conduction: a preclinical canine study. Med Sci Monit 2024; 30:e945007.
131. Amin R, Duytschaever M, Knecht S, le Polain de Waroux JB. Return of the His: a case of atrioventricular node ablation using pulsed field energy. Eur Heart J Case Rep 2025;9:ytaf520.
132. Shen C, Bai R, Jia Z, et al. Unexpected transient atrioventricular block and slow junctional rhythm using pulsed field ablation for slow pathway modification: excited or cautious for ablators. Heart Rhythm 2025;22:1682–1692.
133. van Zyl M, Ladejobi AO, Tri JA, et al. Reversible atrioventricular conduction impairment following bipolar nanosecond electroporation of the interventricular septum. JACC Clin Electrophysiol 2021;7:255–257.
134. van Zyl M, Ladas TP, Tri JA, et al. Bipolar electroporation across the interventricular septum: electrophysiological, imaging, and histopathological characteristics. JACC Clin Electrophysiol 2022;8:1106–1118.
135. Koruth JS, Kawamura I, Reddy VY. Selective sparing of Purkinje fibres with pulsed-field myocardial ablation. Europace 2023;25:330.
136. Amorós-Figueras G, Casabella-Ramon S, Moreno-Weidmann Z, Ivorra A, Guerra JM, García-Sánchez T. Dynamics of high-density unipolar epicardial electrograms during PFA. Circ Arrhythm Electrophysiol 2023;16:e011914.

137. Cespón-Fernández M, Pannone L, Sieira J, et al. Feasibility of reversible electroporation mapping in human atrial flutter. Heart Rhythm 2025. Epub ahead of print.
138. Terricabras M, Lombergar P, Escartin T, et al. Modified unipolar return pulsed field ablation in ventricular myocardium. Circ Arrhythm Electrophysiol 2025; 18:e014006.
139. Meininger GR, Neal RE 2nd, Hunter DW, Krimsky WS. Absence of arrhythmogenicity with biphasic pulsed electric fields delivered to porcine airways. Ann Biomed Eng 2024;52:1–11.
140. Kumru HT, Mattison L, Tarakji KG, Verma A, Sigg DC. Arrhythmogenicity of monophasic and biphasic PFA waveforms in a porcine model. J Cardiovasc Electrophysiol 2024;35:2487–2490.
141. Verma A, Maffre J, Sharma T, Farshchi-Heydari S. Effect of sequential, colocalized radiofrequency and pulsed field ablation on cardiac lesion size and histology. Circ Arrhythm Electrophysiol 2025;18:e013143.
142. Verma A, Feld GK, Cox JL, et al. Combined pulsed field ablation with ultra-low temperature cryoablation: a preclinical experience. J Cardiovasc Electrophysiol 2023;34:2124–2133.
143. Siemer C, Toepfl S, Heinz V. Inactivation of *Bacillus subtilis* spores by pulsed electric fields (PEF) in combination with thermal energy – I. Influence of process- and product parameters. Food Control 2014;39:163–171.
144. Edelblute CM, Hornef J, Burcus NI, et al. Controllable moderate heating enhances the therapeutic efficacy of irreversible electroporation for pancreatic cancer. Sci Rep 2017;7:11767.
145. Edelblute CM, Guo S, Hornef J, et al. Moderate heat application enhances the efficacy of nanosecond pulse stimulation for the treatment of squamous cell carcinoma. Technol Cancer Res Treat 2018;17:1533033818802305.
146. Donate A, Bulysheva A, Edelblute C, et al. Thermal assisted in vivo gene electrotransfer. Curr Gene Ther 2016;16:83–89.
147. López-Alonso B, Polajžer T, Reberšek M, Sarnago H, Lucía Ó, Miklavčič D. Study on effect of electroporation combining high- and low-frequency harmonics. Bioelectrochemistry 2025;165:108971.
148. Petrella RA, Fesmire CC, Kaufman JD, Topasna N, Sano MB. Algorithmically controlled electroporation: a technique for closed loop temperature regulated pulsed electric field cancer ablation. IEEE Trans Biomed Eng 2020; 67:2176–2186.