



Pulsed field ablation: Disrupting technology in cardiac electrophysiology

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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

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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 atriopharyngeal 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

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

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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 (≤ 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 supra-physiological 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.

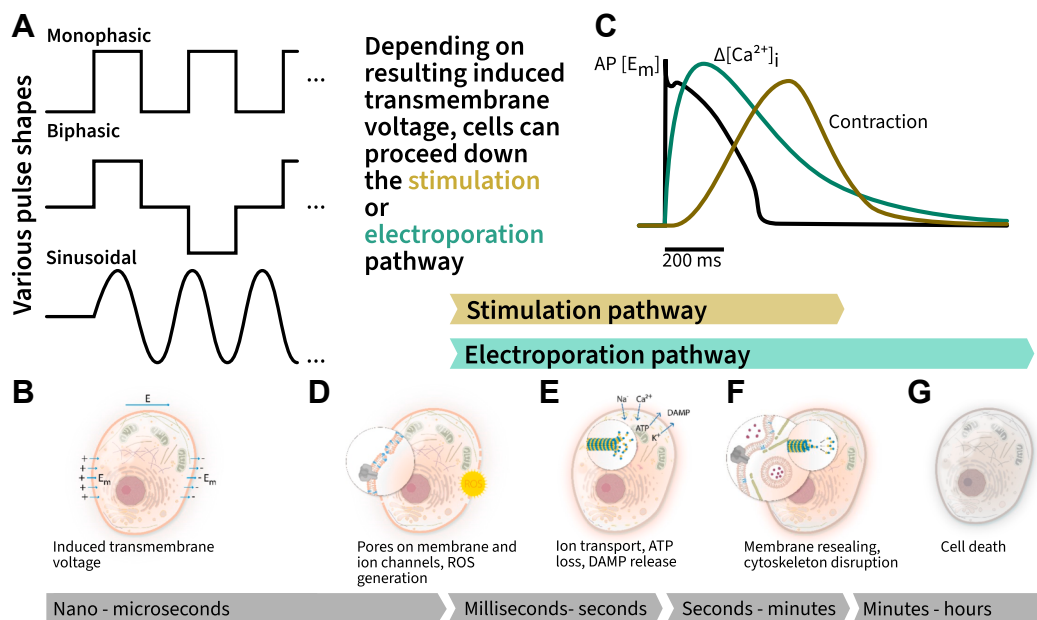


Figure 1

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 μ s for cardiomyocytes), the membrane can fully charge and reach its maximum value. For shorter pulses, the membrane does not fully charge

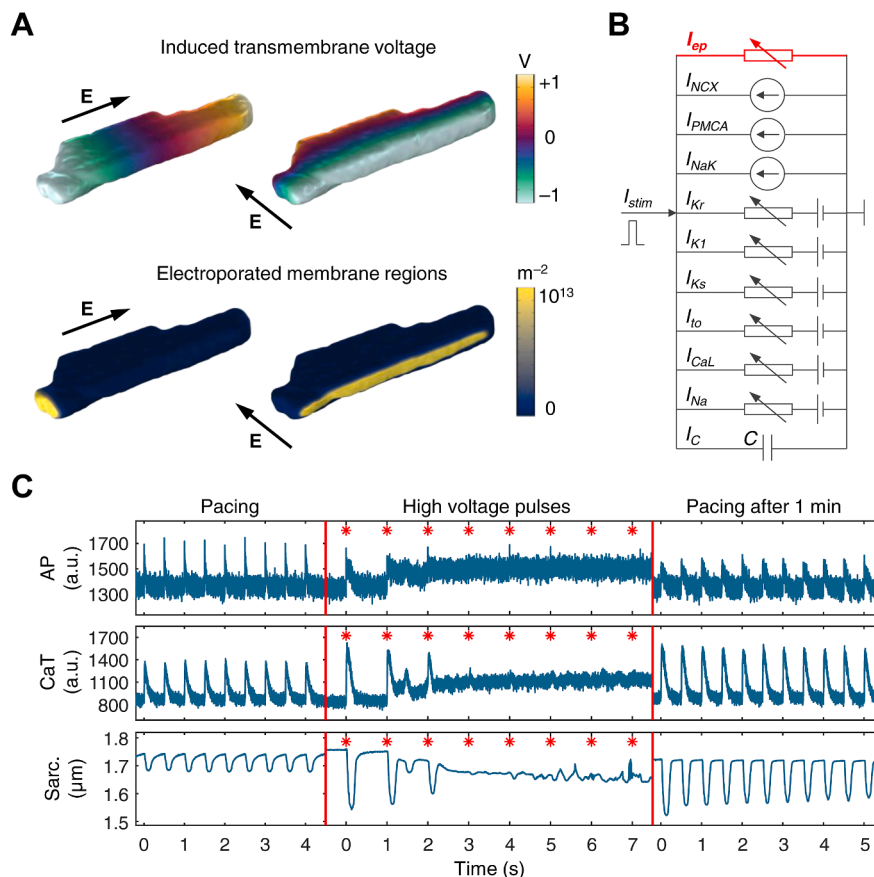


Figure 2

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

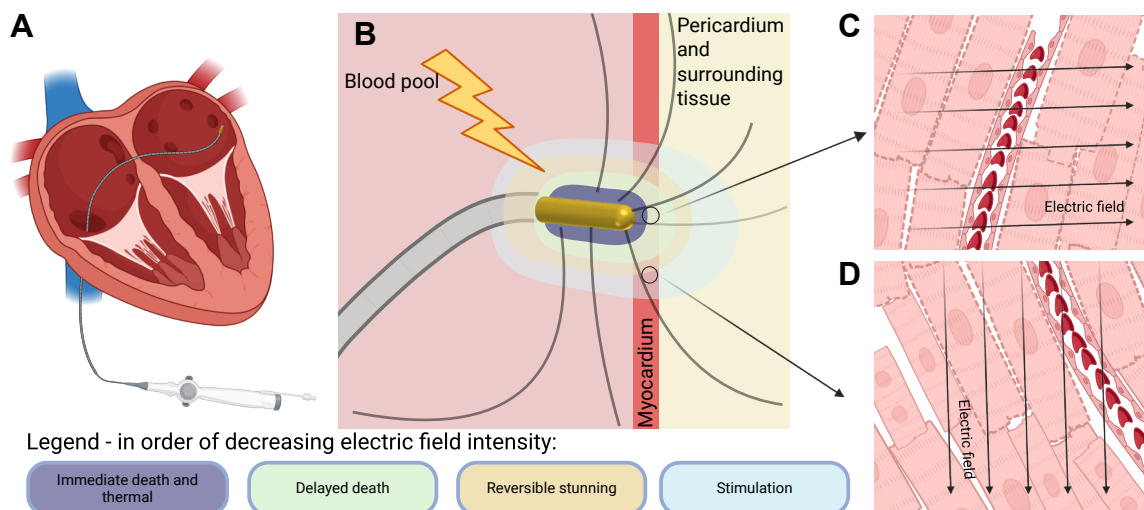


Figure 3

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 μ s 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 transmural-ity 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 electrochemistry⁷⁶ 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 signals⁷⁸ 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

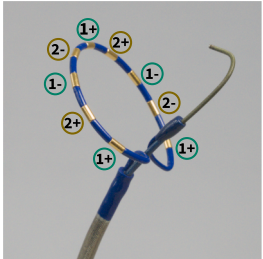
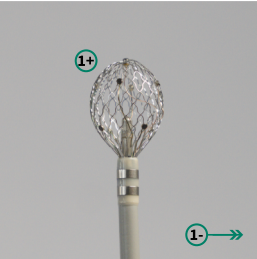
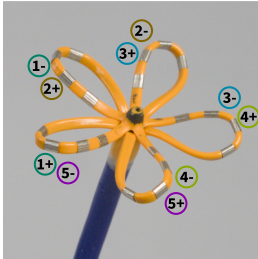
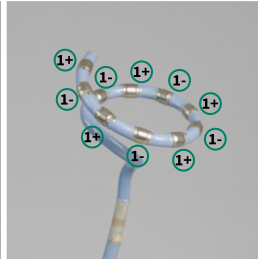

Circular loop catheter	Lattice-tip catheter	Pentaspline catheter	Variable loop catheter	Spherical array catheter
				
Amplitude: 1500 V	Amplitude: <2000 V; 24–32 A	Amplitude: 1800–2000 V	Amplitude: 1800 V	Amplitude: 1700 V
Vectoring: Bipolar	Vectoring: Monopolar	Vectoring: Bipolar	Vectoring: Bipolar	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 device¹²⁰ (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 flow¹²⁵ 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 patients⁷⁵ 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 reported¹³⁷; 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 PFA¹⁴¹ 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.

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