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Review

Pulsed Field Ablation for the Treatment of Atrial Fibrillation: A Review and a Look into its Future

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Abstract

Pulsed field ablation (PFA) is a novel technology to treat atrial fibrillation (AF) utilizing electric fields to induce nonthermal irreversible electroporation of electrically active cardiac tissue to induce cardiac cell death. PFA offers improved safety benefits compared to traditional radiofrequency ablation (RFA) and cryoablation by specifically ablating only cardiac tissue. However, there are avenues for further optimization including neurological risk associated with microbubble formation and left atrial function post ablation. Various PFA devices with different electric pulse waveforms have been studied and tested in human trials, with the majority utilizing microsecond duration pulses. Shorter nanosecond duration pulses, or nanosecond PFA, is beginning to be studied for AF ablation. In this review we will delve into current waveforms used for PFA, areas for improvement, mechanisms behind nanosecond PFA, and its clinical impact for cardiac ablation.

Keywords

arrhythmia; catheter ablation; atrial fibrillation; pulsed field ablation; nanosecond; electroporation

Introduction

AF is the most common arrhythmia seen in clinical practice, which left uncontrolled can lead to devastating consequences such as stroke and heart failure [1]. In patients refractory to rate control or anti-arrhythmic medication, catheter ablation of the electrically active tissue around the pulmonary veins can reduce arrhythmia burden [2]. The main modes of ablation in clinical practice today are radiofrequency ablation (RFA) or cryoablation, which rely on thermal heating or cooling to destroy tissue. PFA is an emerging technology that is a non-thermal ablation method that relies on creating permanent pores in the cell membrane of cardiomyocytes [3–5]. PFA may be safer than RFA with lower risk of complications such as esophageal lesions, pulmonary vein stenosis, and nerve injury, in part due to the specificity of PFA for ablating cardiomyocytes over other cell types [6–8]. The parameters of the electric pulse utilized have an important role in the effect of PFA on tissue. This review will discuss the waveforms utilized by current PFA devices tested clinically, and dive into the emerging research on ultra-short nanosecond duration PFA.

PFA Waveform Parameters

An electric field has multiple properties that can be varied to cause differential effects on tissue. Electric stimulation is applied in a pulsed manner usually in the form of a square wave, but other waveforms such as a sinusoidal pattern are also possible [3]. The stimulation can be a monophasic configuration where voltage pulses are continuously positive, or a biphasic configuration where the voltage pulse has a positive region and a negative region [9,10]. The amplitude of the pulse can be altered to change the field strength, quantified in Volts (V)/meter (m), and the duration of the pulse can be altered by changing the pulse width [11]. A pulse train can be formed by modifying the inter-pulse interval or frequency of pulses to determine the total number of pulses delivered to the tissue [10]. Further characteristics such as the positioning of the electrode from which the field is emitted and distance to the tissue can also alter the effects on the target [12]. Current PFA devices in clinical testing and use typically employ microsecond-scale pulse widths (Fig. 1A); nanosecond PFA entails utilizing much shorter pulse widths at the expense of greater voltages to perform ablation (Fig. 1B). The details of current clinical devices will be discussed first, followed by review of research into the more nascent nanosecond domain.

Current PFA Clinical Devices and their Waveforms

A PFA delivery system requires a generator that can produce the pulse waveform as well as a catheter with electrodes for application of electric fields. There are multiple PFA devices currently on the market, each utilizing their own specified pulse parameters and, in most cases, their own custom catheters [7,8,13–16] (Table 1, Ref. [7,8,13–19]). Although the exact details of the pulse waveform used by these devices is proprietary, all of these devices

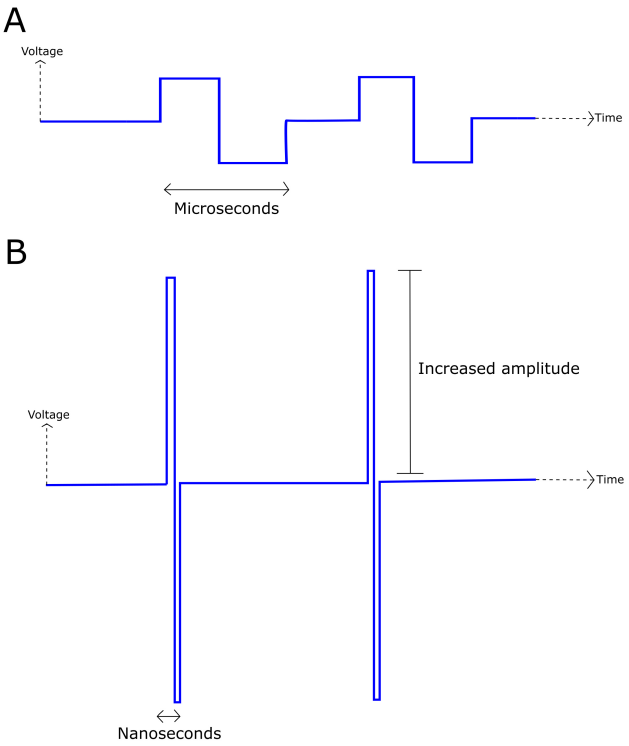

Fig. 1. Schematic depictions of PFA waveforms (A) A biphasic waveform with two pulses of microsecond duration (B) A biphasic waveform with shorter, nanosecond duration pulses. The voltage is increased due to the shorter pulse width. PFA, Pulsed field ablation.

are similar in that the pulse width of stimulation is in the microsecond or greater order of magnitude. Initial studies tested both monophasic and biphasic waveforms [7]. However, monophasic waveforms have been shown to trigger greater skeletal muscle contraction requiring general anesthesia, while biphasic waveforms carry the advantage of reduced contraction and ability to perform ablation under conscious sedation [7,20].

The initial trial of PFA in 2018 on 22 patients with paroxysmal AF (pAF) tested both endocardial and epicardial ablation using a pulsed waveform of millisecond duration, delivered in trains over a few seconds [17]. The voltage was the only varied parameter, with endocardial ablation using 900–1000 V and epicardial ablation using 2100–2400 V. In a subsequent larger study of 81 patients in the IMPULSE and PEFCAT trials, the authors performed pulmonary vein isolation (PVI) using shorter microsecond-scale pulses delivered in a bipolar fashion with both monophasic and biphasic waveforms tested [7]. Voltages in this study were between 900 to 1000 V for monophasic and 1800 to 2000 V for biphasic waveforms respectively. The authors further optimized the biphasic waveform, although the specifics were not able to be disclosed due to proprietary information. PFA was delivered using the Farapulse™ System, from Farapulse Inc. (Menlo Park, CA, USA) now acquired by Boston Scientific Inc., which utilizes a pentaspline catheter deployable in a flower or basket configuration. Extending this approach to persistent AF (persAF), the PersAFOne trial carried out PFA for PVI as well as left atrial posterior wall ablation [18]. The waveforms in this trial also employed microsecond-scale biphasic pulses at 1600 to 2000 V.

Based on these first in human studies, Schmidt *et al.* [19] described the adoption and usage of PFA technology in Germany in an all-comer group of 191 AF patients in the 5S study. Utilizing the Farapulse™ ablation system approved in Europe, they followed the biphasic microsecond-scale protocol of previous trials. Voltage was also kept at 1800 to 2000 V and a train of 5 consecutive waveforms was delivered for a total application time of 2.5 seconds. A survey of 24 centers in Europe that adopted PFA after Farapulse™'s approval covered 1758 patients with a mixture of pAF and persAF, and also utilized the same standard waveform protocol [21].

Other PFA devices have also been developed with a different catheter shape and their own individualized waveform. The Multi-Channel PFA Generator from Biosense Webster Inc. (Irvine, CA, USA) is compatible with the electroanatomical mapping system CARTO3. In the inSPIRE trial this system was used to treat 226 patients with pAF, employing a square wave biphasic waveform with microsecond-width pulses at 1800 V delivered in trains for a total 250 milliseconds per application [13]. A sphere-shaped lattice-tipped catheter allowing for toggling between PFA, RFA, and electroanatomic mapping, the Sphere-9 developed by Affera Inc. (Watertown, MA, USA) which was then acquired by Medtronic (Minneapolis, MN, USA), has also been studied for ablation of pAF or persAF [14]. This device also delivers biphasic microsecond-width pulses, in a train of 3–5 seconds. Notably the catheter also irrigates the delivery area with saline. In a study of 178 patients with pAF or persAF, various waveforms were developed to attempt to increase electrical isolation efficiency [14]. The first evolution PULSE1 used 3–5.5 second trains of lesions with saline irrigation at 4–30 mL/min, the second-generation PULSE2 used 4 second trains with saline at 15 mL/min, and the final generation PULSE3 also used 4 second trains and 15 mL/min. The exact parameters of the waveform and the difference between PULSE2 and PULSE3 is kept proprietary. However, these waveform characteristics have a great impact on tissue effects, as the durability of PVI at an average of 3 months post-ablation improved from 32% of patients with all pulmonary veins still isolated with PULSE1 to 64% with PULSE2 and to 90% with PULSE3.

Further evidence for the importance of waveform parameters comes from the ECLIPSE-AF study of the CENTAURI system for PFA ablation, developed by Galvanize Therapeutics Inc. (San Carlos, CA, USA) [15]. The CENTAURI system has a generator that is compatible with three

Table 1. Current PFA devices undergoing clinical testing or use.

Device	Catheter structure	Study	Pulse width
Farapulse™ System from Boston Scientific Inc.	Pentaspline catheter deployable in flower or basket-shaped configuration	Reddy *et al*. 2018 [17] Reddy *et al*. 2019 [7]	Initial studies: Millisecond scale
		Reddy *et al*. 2020 [18] Schmidt *et al*. 2022 [19]	Refined waveform: Microsecond scale
Multichannel PFA Generator from Biosense Webster Inc.	Variable loop circular catheter	Duytschaever *et al*. 2023 [13]	Microsecond scale
Sphere-9 from Affera Inc. (acquired by Medtronic)	Spherical lattice-tipped catheter	Reddy *et al*. 2023 [14]	Microsecond scale
CENTAURI™ system from Galvanize Therapeutics Inc.	Generator that is compatible with existing commercial ablation catheters	Anić *et al*. 2023 [15]	Millisecond scale
PulseSelect PFA System from Medtronic	Annular electrode array catheter	Verma *et al*. 2023 [8]	Undisclosed pulse width
Globe® from Kardium Inc.	Spherical electrode array catheter	Turagam *et al*. 2023 [16]	Microsecond scale

different commercially available ablation catheters (Abbott TactiCath SE, Boston Scientific StablePoint, and Biosense Webster ThermoCool ST). As the workflow was optimized through the trial, patients ended up in 5 cohorts with different waveform settings or catheters used in each group. Waveforms ranged from 1.4 to 3.4 milliseconds duration, currents from 19 Amps to 25 Amps, and lesion diameter was varied. Similar to the Sphere-9 trial, the first two cohorts had low 90-day PVI durability while later cohorts with optimized parameters showed increased durability. Medtronic has also developed a catheter with a circular array of electrodes, the PulseSelect PFA System, tested in the PULSED AF trial [8]. Ablation is delivered in biphasic pulse trains of 100–200 milliseconds, with a voltage around 1500 V. The exact pulse width in the train is not disclosed. Finally, the Globe is a spherical electrode array PFA catheter from Kardium Inc. (Burnaby, British Columbia, Canada) that was tested for PVI [16]. Interestingly, in this study patients received microsecond-width pulses at 1700 V in either a single application or three applications repetitively at the same site. Repetitive application led to more durable PVI, with 30% of single application patients showing persistent complete PVI at 2–3 months versus 100% in the multiple application group. Therefore, in addition to the individual parameters of each pulse, the total amount of applications can also be varied to achieve the desired outcome. While a single application would be most time efficient, an ablation method that has greater safety but requires multiple applications may be worthwhile. The clinical studies discussed above using PFA with microsecond or greater duration pulses have shown promising safety data and may lower the risk of complications seen with conventional ablation such as pulmonary vein stenosis and esophageal lesions [8]. However, there are still areas where PFA's safety profile may be improved.

Microbubble Formation and Neurological Risk during PFA

Ablation procedures have a low rate of neurological complications such as stroke, however studies have shown a significant portion of patients show silent neurological findings on magnetic resonance imaging (MRI) after ablation that may be attributable to air or thrombus embolization [22]. MRI findings can be categorized as silent cerebral events (SCE) that are diffusion-weighted imaging (DWI) positive, or silent cerebral lesions (SCL) that are DWI positive as well as fluid-attenuated inverse recovery sequence (FLAIR) positive [23]. While SCLs have more evidence associated with histological changes and scar formation, the FLAIR changes cannot be detected until 2–7 days post procedure [24]. SCEs are a more sensitive marker that only rely on DWI, and thus are around three times as common as SCLs, however their pathological significance is less clear [23]. The rate of SCE with traditional RFA in the literature is around 6.8–24% and for SCL between 7.4–8.3%, and appears to vary with the ablation technology used [23]. For example, cryoballoon ablation shows lower rates of SCE between 8.9–18% and SCL between 4.3–5.6% [23]. This raises the question of how PFA fares compared to current technology, and whether the waveform parameters utilized may alter the incidence of neurological lesions.

In the IMPULSE and PEFCAT trials the authors typically observed ultrasonic microbubbles on intracardiac echocardiography immediately after PFA application, hypothesized to be due to electrolysis of water in the presence of an electric field [7]. However, they demonstrate that no patients presented with evidence of stroke, transient ischemic attack (TIA), or systemic embolism, and additionally performed MRIs on 13 patients that did not show evidence of silent ischemic events. A further combined analy-

sis with the results of the PEFCATII trial showed 16 out of 18 patients showed no MRI lesions after PFA; one patient with a history of TIA's experienced brief dysphasia after the procedure with a small correlate lesion found on DWI but not FLAIR SCE, and a second patient also showed a DWI positive lesion SCE [25]. The 5S study also carried out MRI in a subset of 53 patients, 24–48 hours after the ablation. In the initial validation stage of this trial, the PFA catheter's electrograms were verified with a circular mapping catheter, requiring catheter exchange. After the first 25 patients the study transitioned to the streamline phase where the circular mapping catheter was omitted. During the validation stage two patients developed strokes after the PFA procedure: the first with blurred vision and novel ischemic lesion on MRI, and the second with right hand weakness and gait ataxia with multiple small acute lesions in the basal ganglia and cerebellum. In both patients the symptoms had resolved at later follow-up. During the streamline phase no patients had neurological symptoms, suggesting that the catheter exchange may have played a role. However, diffusion weighted MRI showed asymptomatic cerebral injury in 10/53 (19%) of patients albeit without neurological deficits [19].

An alternate PFA system developed by Biosense Webster Inc. in the inSPIRE trial also underwent testing for brain ischemia using MRI [13]. In this study an initial 4 out of 6 patients showed SCLs, leading to workflow modifications such as instating a 10 second pause between PFA applications, minimizing catheter exchanges, and ensuring anticoagulation adherence. Further results showed SCL in 4 out of 33 patients (12%). The authors note all the lesions were asymptomatic and resolved by a 3 month follow up. The Sphere-9 system that combines PFA, RFA, and electroanatomic mapping showed SCEs in 5 (9.8%) and SCLs in 3 (5.9%) of 51 patients in their initial study [18]. A second larger study of the device showed SCEs in 7 (7.9%) and SCLs in 6 (6.7%) out of 89 patients [14]. With Medtronic's circular catheter, the PulseSelect™ system tested in the PULSED AF trial, 1 out of 150 pAF patients and 0 out of 150 persAF patients developed stroke post PFA [8]. Further, MRI studies of a 45 patient cohort showed 4 (8.9%) had postprocedural SCLs while SCEs were not reported. Functional testing of this cohort was also carried out with Mini Mental State Examinations, which did not significantly differ from before PFA to 30 day follow up.

Interestingly in the ECLIPSE-AF trial testing the CENTAURI PFA system, monitoring for microbubbles with intracardiac echocardiography during the index procedures did not demonstrate any microbubbles in 61 procedures [15]. Furthermore, MRI of 36 patients showed SCE in 4 (11.4%) and SCL in 0 (0%) of patients. The four patients with SCEs were all in cohort 4 of the study where the Boston Scientific Inc. StablePoint catheter was used. Similar to other studies all patients were asymptomatic, and lesions resolved at 30 day follow up. Finally, the PULSE-EU study of the Kardium Inc. Globe system that tested single versus multiple applications of the same PFA waveform showed SCEs in 2 patients (28.6%) in the single application group and 1 patient (11.1%) in the multiple application group [16]. This suggests additional PFA applications do not increase the number of brain lesions, although sample sizes are small.

The overall rate of clinically symptomatic neurological deficits after PFA appears to be low, as confirmed by the EU-PORIA real-world outcomes study of the Farapulse system in Europe, which finds TIA or stroke in 7 out of 1233 patients (0.6%) [26]. The only randomized controlled trial of PFA recently compared the Farapulse system from Boston Scientific against traditional RFA or cryoballoon ablation for pAF and demonstrated non-inferiority in efficacy as well as serious adverse events at 1 year [27]. In this study, there were no clinical strokes and 1 TIA seen in the 305 patients treated with PFA, while there was 1 stroke and no TIAs seen in the 302 patients treated with thermal ablation. Thus, there appears to be a low rate and incomparable difference in symptomatic neurological deficits from these different ablation modalities. A brain MRI on a subset of patients however showed 3/33 (9%) treated with PFA had asymptomatic ischemic phenomena (SCE or SCL) compared to 0/37 treated with thermal ablation. It is unclear whether these asymptomatic neurological lesions are associated with long-term effects on cognition [23]. Regardless a method of ablation that could minimize the formation of these lesions would be ideal. The lack of any microbubbles or SCLs with the CENTAURI system compared to other PFA systems suggests that there are parameters that can be modified to prevent their development.

Left Atrial Function Post-Ablation with PFA

The left atrium (LA) has important functions that are perturbed in AF and that may be differentially altered by ablation modality. The LA's hemodynamic function can be divided into reservoir, conduit, and booster phases [28]. The LA acts as a reservoir during ventricular systole, enlarging without increasing chamber pressure to prevent pulmonary hypertension. It acts as a conduit between the pulmonary veins and the left ventricle (LV) during ventricular diastole, and its contraction at end-diastole boosts LV filling. Furthermore, the LA also plays a neurohormonal role, with atrial stretch triggering local natriuretic peptide release, as well as nerve activation leading to inhibition of central sympathetic outflow [29]. AF is associated with deleterious structural and functional changes including LA enlargement and reduced atrial systolic function [30,31]. Prolonged AF leads to progressive LA fibrosis that reduces LA capacitance and thus reservoir function [32].

The effect of conventional ablation on LA function has conflicting results in the literature. Mechanistically restoration of sinus rhythm via ablation may boost systolic function, while the creation of scar tissue from ablation may counteract this benefit and lead to reduced atrial distensibility. Electron beam tomography on pAF patients after RFA revealed left atrial edema in a large portion of patients [33]. Furthermore, the severity of the edema depended on the extent and amount of radiofrequency energy delivered, although the edema naturally resolved with time. Long term follow-up of AF patients after RFA via echocardiography has shown improvement in LA size as well as LV diastolic and systolic function [34]. On the other hand, contrast enhanced computed tomography (CECT) study of pAF patients by Lemola *et al*. [35] demonstrated that LA systolic function, measured via LA emptying fraction (LAEF), declines after ablation. In contrast, Verma *et al*. [36] performed echocardiography and cine electron beam computed tomography (EBCT) on pAF and persAF patients after pulmonary vein antrum isolation and noted no adverse effect on LA function and even improvement in LAEF at 6 months post-ablation. The authors note to have a larger sample of patients than Lemola and colleagues [36]. However, a later study by Wylie and colleagues [37] that used cardiovascular magnetic resonance imaging (CMR) of pAF and persAF patients revealed lower LAEF after ablation. Furthermore, the authors studied patients who underwent limited pulmonary vein ostial ablation, and surmised that more extensive circumferential ablation with linear lesions to the LA posterior wall, roof and/or mitral isthmus may have an even greater effect on function. Given the numerous conflicting studies, a meta-analysis of the effect of catheter ablation found that while left atrial size is improved, LAEF decreases in pAF patients and is not significantly altered in persAF patients [38]. Therefore, conventional ablation appears to have a detrimental effect on LA systolic function in at least a subset of patients [29]. RFA may also in rare cases cause LA diastolic dysfunction severe enough to lead to pulmonary hypertension and dyspnea, known as stiff LA syndrome [29,39]. This complication occurred in 1.4% of patients in one study and was more likely to occur in patients with severe LA scarring [39].

The clinical significance of reduced atrial function is not well established. However, reduced LA contractile function is predictive of AF recurrence after catheter ablation [40,41]. While it is unclear if improving LA function after ablation will lead to greater long-term success of cardioversion, methods of ablation that preserve LA function would be ideal. Initial results comparing PFA with RFA and cryoablation showed less late gadolinium enhancement at 3 months post-ablation, suggesting less chronic fibrosis, and greater recovery of LA reservoir and booster pump function with PFA [42]. Further work will be needed to confirm these results in a wider patient pool, as well as study the effect of different pulse parameters on LA function. Nanosecond PFA that allows for reduced total energy delivery to the tissue may better preserve LA function and reduce the risk of complications such as stiff LA syndrome. More studies need to be performed to evaluate this.

Is Nanosecond PFA The Future?

Pulsed electric fields have been extensively studied in non-cardiac cells for applications ranging from laboratory transfection to tumor cell killing [43]. The mechanistic findings from the wider body of non-cardiac research is presented first, followed by insights from cardiac specific studies.

The principal mechanism behind PFA is electroporation of the cell membrane, leading to the formation of pores that allow for free flow in and out of the cell and apoptosis [44,45]. In order for pore formation to occur, the magnitude of the electric field across the cell membrane must reach a certain strength [46]. The magnitude of the electric field also determines whether the pores are formed transiently, leading to reversible electroporation that is commonly used in laboratories for transfection, or permanently, leading to irreversible electroporation and cell death [46]. Microsecond and millisecond duration electric field pulses lead to movement of ions in the extracellular and intracellular space, with the cell membrane acting as a barrier that leads to capacitive charging. This phenomenon amplifies the externally applied electric field, known as the Maxwell-Wagner effect, such that the critical magnitude needed across the cell membrane for pore formation is reached [47,48]. Pores have been observed to preferentially form at the poles of cells that face electrodes since they experience the greatest ion alignment [48–50].

Shorter nanosecond-duration pulsed electric fields (nsPEF) are also capable of inducing cell death [51–53]. Nanosecond fields are too brief to allow for capacitive charging to amplify the external electric field across the cell membrane [52,54]. Modeling and experimental results demonstrate that nanosecond electric pulses are capable of charging the smaller intracellular membranes of organelles, allowing for electroporation of structures such as the nucleus, mitochondria, and endoplasmic reticulum, which may contribute to apoptosis [55]. Furthermore, it was shown that electroporation of intracellular membranes could occur without electroporation of the outer cell membrane in COS-7 cells using nsPEF [56]. However, follow-up studies on a wider range of cell types showed that nsPEF are indeed capable of causing pore formation on the plasma membrane [57–60]. The pores formed from nsPEF may be smaller in size than the pores from microsecond and millisecond duration fields, at around 1–1.5 nanometers, and thus not detectable in experiments by large dyes such as propidium iodide that could not pass through them [61]. Nanosecond electric fields thus appear capable of creating a

transmembrane potential that is enough to electroporate the cell membrane, but due to the lack of amplification from capacitive charging require a higher external field strength to do so [48,62]. Although a higher voltage is used, the shorter duration of the pulse means the total energy delivered is often lower with nsPEF. Pores also form more uniformly across the cell rather than at the poles [48,54].

Pulsed electric fields may also lead to cell death via migration of membrane phosphatidylserine, a phagocytic signal on apoptotic cells, from the cytosolic to the extracellular side of the membrane via pores. This process has been demonstrated to occur in response to both microsecond and nanosecond duration fields [63]. Comparison of 10 nanosecond, 300 nanosecond, 1.8 microsecond, and 9 microsecond pulses in cells showed that the 10 nanosecond pulses were the least efficient at triggering cell death, but more selective between two different cell types [64]. While the 1.8 microsecond pulses led to pores large enough for propidium iodide uptake, the 10 nanosecond pulses did not, however both pulses resulted in phosphatidylserine externalization. Nanosecond fields may thus have the advantage of even greater selectivity than microsecond fields, although more pulse applications may be required for effective ablation given the lower efficiency of cell killing. In addition to nanopore formation of the cell membrane and organelles and phosphatidylserine externalization, other mechanisms of action for nsPEF have also been described. Membrane cholesterol concentration and lipid rafts appear to be an important determinant of nsPEF response, as is oxidation of membrane lipids [65,66]. Further research is needed on these effects of nsPEF and comparison with microsecond and millisecond fields.

The above-described insights on nsPEF action come from the wide body of literature studying their effect on non-cardiac cell types. For application to AF ablation, it is important to understand the mechanistic effects of nsPEF on cardiac cells. Nanosecond pulsed fields have been shown to similarly affect cardiomyocytes by creating pores in the sarcolemma, allowing for calcium flow independent of ion channels [67]. Paralleling non-cardiac cell results, nsPEF requires greater voltages to achieve electroporation in cardiomyocytes compared to microsecond fields. In a detailed study on isolated mouse, pig, and rabbit cardiomyocytes, Semenov and colleagues compared 10 microsecond, 800 nanosecond, and 200 nanosecond width shocks [68]. The authors initially identified the voltage that was necessary to trigger calcium transients in the cell for each pulse duration, then used five times that voltage for electroporation. At the 5 × voltage the nanosecond pulses demonstrated significantly reduced electroporation compared to the microsecond pulses. The authors note this benefit of nsPEF for defibrillation, which would reduce unnecessary electroporation and cardiac damage. However, for the purposes of ablation, this suggests that increased voltage parameters may be necessary to achieve the same degree of electroporation with nsPEF. It is worth noting that electroporation was determined in this study by the uptake of propidium iodide, a membrane-impermeable dye that fluoresces red upon binding nuclear material. However, this dye molecule may be too large to enter and detect pores that are exceedingly small such as nanopores formed by nsPEF. Exposure of rat embryonic cardiomyocytes to 10 nanosecond versus 4 millisecond duration fields showed that at pulse parameters that led to the same level of calcium influx, the millisecond fields led to more propidium iodide uptake, suggesting that millisecond fields create larger membrane pores while nsPEF creates smaller pores [48]. Furthermore, calcium imaging revealed influx beginning at the poles of the cells with millisecond fields, while nsPEF led to homogenous influx across the cell. This supports results discussed earlier in other cells that nsPEF leads to more uniform electroporation across an individual cell.

A key feature of cardiomyocytes is their rod-shaped structure. Pulse width has interestingly been shown to determine whether the orientation of cardiomyocytes within the electric field affects electroporation. In nanosecond duration pulses, cardiomyocytes that are oriented perpendicular to the field are more electroporated than cells oriented parallel [69]. However, in the microsecond pulse domain cell orientation does not have an effect on electroporation efficacy. Furthermore, at even longer millisecond pulse durations cells oriented parallel to the field are more electroporated than those perpendicular. These results suggest that while nsPEF cause pore formation uniformly across a cell, on a tissue level cells that are oriented perpendicular to the applied field will be more likely to be targeted. Microsecond pulses may carry the advantage of more thorough ablation in tissue with differently oriented cells. However, with advancements in understanding of the myofiber architecture in the atrium the orientation selectivity of nsPEF may become useful for ablating specific tracts [70]. Additionally, catheters may be designed to apply multidirectional electric fields if complete ablation of all orientations is desired. It is worth noting these studies were carried out in H9c2 rat ventricular myoblasts and AC16 human ventricular myocytes in culture [69]. Given the complex three-dimensional nature of the atrium and factors such as extracellular matrix, vessels, and nerves that alter structure, further studies will be needed to confirm this phenomenon *in vivo*, not just in cell culture.

Non-Cardiac Clinical Studies of Nanosecond PFA

While nanosecond pulsed fields have not yet been studied in humans for cardiac ablation, they are beginning to be studied in the field of oncology. Different duration pulsed fields including millisecond, microsecond, and nanosecond fields have all been researched for tumor ablation and have been reviewed previously [71–73]. Mi-

crosecond pulses have been applied for direct killing of tumor cells via irreversible electroporation, as well as in electrochemotherapy where the electric field is used to increase tumor cell permeability to chemotherapeutic drugs. Nanosecond pulses are an emerging area within tumor treatment as well. While microsecond duration pulses in tumors appear to lead to cell death via necrosis, nanosecond duration pulses may favor apoptosis, possibly due to smaller pore size formation that only allows ion fluxes while maintaining adenosine tri-phosphate (ATP) that is necessary for apoptosis [74].

The first human trial of nsPEF was carried out for basal cell carcinoma and ablated 10 lesions in three patients [75]. Electric field pulses 100 nanoseconds wide were delivered at an amplitude of 30,000 V/cm. The results demonstrated that 7 of the 10 treated lesions were completely devoid of basaloid cells, two partially regressed, and only one lesion recurred as squamous cell carcinoma by week ten. The treatment led to edema and crust formation, followed by the gradual return of normal skin appearance. Importantly, no visible scars were observed at the treated sites. Initial trials in humans have also demonstrated the feasibility of nsPEF for the treatment of other cutaneous lesions such as seborrheic keratosis and sebaceous gland hyperplasia [76,77]. Clinical studies on applications for visceral organs are limited; however, Liu and colleagues [78] have trialed nsPEF for treating hepatocellular carcinoma (HCC) in 15 patients [79]. Using 300 nanosecond duration pulses at 30,000 V the authors were able to ablate liver tumors within 0.5 cm of critical structures such as the portal vein, hepatic vein, biliary tree, or gastrointestinal tract. These results are encouraging for translation to cardiac applications.

Cardiac Studies of Nanosecond PFA

Although there have been limited human trials of nsPEF for treatment of tumors, there are no clinical studies applying nsPEF to the heart. Current insights are limited to research performed in pre-clinical animal models. Xie and colleagues [80] applied nsPEF to rabbit hearts in an *ex-vivo* Langendorff perfusion setup. The nsPEF were delivered via two electrodes that were inserted transmurally into the ventricle, and the waveform used a pulse width of 350 nanoseconds applied in different pulse trains and amplitudes. Optical mapping was carried out to assess the effects on heart conduction, and successfully confirmed that electrical block could be achieved consistently at 2300 V when electrodes were 2.3 mm apart and 4000 V when 4 millimeters apart. A single pulse was not sufficient for ablation and multiple pulses in a train were needed. Notably the authors state that their nanosecond ablation approach uses a thousand times less energy than the microsecond pulses in the seminal PFA studies of Lavee and colleagues [44]. The nanosecond pulses use higher field strengths but at a much lower duration. Further studies from the same group have shown efficacy of nsPEF at 10,000–12,000 V for ablation in pig atria and ventricular myocardium with transmural penetration, albeit with a surgical approach for electrode positioning [81]. Catheter-based delivery would be the primary modality of ablation in clinical practice. There has been initial work on nanosecond PFA delivery utilizing a circular multielectrode catheter, the CellFX system from Pulse Biosciences Inc. (Hayward, CA, USA), for ablation in pigs [82]. Ablation was attempted at the superior vena cava, right superior pulmonary vein, and discrete sites on the atria, with successful delivery at 22/22 sites and histology showing transmural cell necrosis in 21 of 22 lesions. These data were published in a recent abstract and more detailed findings will be insightful.

Nanosecond PFA has also begun to be studied for ventricular targets. Tan *et al*. [83] utilized the CellFX generator to create nanosecond electric fields, but delivered the stimulation using conventional transvenous pacemaker leads, either with a single lead in a bipolar manner from helix-to-ring, or using two leads. In their study they targeted ablation to the right ventricular septum of canines, and observed that a single lead was limited in the amount of electric field energy that could be delivered due to arcing, resulting in only mild reduction in cardiac electrogram voltages and no evidence of lesion creation on cardiac MRI or histopathology. The two-lead system allowed for a stronger electric field delivery and showed significant electrogram amplitude decreases, along with associated late gadolinium enhancement on MRI, dense fibrosis, and fewer than 5% residual cardiomyocytes on histology. Interestingly the authors also compared the lesions from nanosecond PFA with RFA on histopathology, and found the RFA lesions showed more necrotic cardiomyocytes interspersed with fibrosis and hemorrhage. This data fits with nanosecond fields promoting apoptosis over necrosis as discussed earlier, and may benefit LA function after ablation. However, the study in pig atria from Koruth *et al*. [82] described transmural cell necrosis at nanosecond PFA sites, which suggests that other parameters besides pulse duration may play a role. Tan and colleagues [83] also note that one of their nanosecond PFA cases showed adherent organizing thrombus formation at the ablation site. This may be due to the screw-in nature of the lead delivering PEF rather than the nanosecond fields, but is a potential risk. Nanosecond fields have also been shown to be capable of transiently blocking the atrioventricular (AV) conduction system in canine hearts when ablation is targeted to the interventricular septum [84]. This effect is dependent on the level of energy delivered and is reversible with time, with histology demonstrating ablation of myocardium and sparing of Purkinje fibers. It is unclear if the transient blocking of the AV conduction system is specific to nanosecond versus microsecond pulses, but is a point of caution when applying pulsed fields to the ventricle during ablation.

Future Directions

Pulsed field ablation has burst onto the electrophysiology stage as an effective method of ablation for AF that is safer than conventional RFA or cryoablation. While there are multiple PFA devices in clinical testing, each with their individual proprietary waveforms, all of them utilize microsecond or greater duration pulses. While generating nanosecond duration electric field pulses was challenging in the past due to their ultrashort and high voltage nature, technological advancements have allowed for nsPEF to become studied more easily [85]. Nanosecond fields differ mechanistically from the microsecond and millisecond fields that have currently been tested in human patients. By creating smaller nanopores in the cell membrane as well as intracellular organelles nsPEF may favor apoptosis over necrosis, and also allow for myofiber orientation specific ablation. Initial results from preclinical models suggest that nanosecond PFA can be a promising treatment for AF. Nanosecond PFA may allow lower energy application that could improve the safety profile of ablation. While the results discussed show that nanosecond PFA can ablate cardiac tissue in porcine, canine, and rabbit models there have not been long-term studies on the durability of electrical isolation. These studies would need to be conducted to confirm the irreversibility of nanosecond PFA. The selectivity of nanosecond pulses for myocardium and sparing of nerves and esophageal tissue also needs to be demonstrated. If confirmed this would open the avenue for studies directly comparing nanosecond versus microsecond PFA for AF ablation in animal models or humans. Other potential barriers to implementation in practice include the number of applications of electric field pulses necessary to successfully ablate tissue with nanosecond versus microsecond fields, which could affect procedure time and time under anesthesia. It remains to be seen if nsPEF leads to lower rates of microbubble formation and cerebral ischemic findings. The impact of nsPEF versus longer pulse durations on LA function after ablation would also benefit from study. Other outcomes such as atrial arrhythmia recurrence could also be compared with different pulse duration parameters. In sum, nanosecond PFA is an area for further exploration with potential for improving the surgical treatment of atrial fibrillation.

Availability of Data and Materials

No original data were required for the preparation of this review.

Author Contributions

EYW and PG contributed to the design of this work. PG and NS drafted the initial manuscript. PG and EYW edited and all authors read and approved the final manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed to editorial changes in the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Not applicable.

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Conflict of Interest

E.Y.W. has been a consultant for Boston Scientific and national PI of Boston Scientific sponsored LUX-DX Trends trial. The authors have no financial interest in the devices described in the manuscript.

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