

Pulsed Field Ablation for the Interventional Treatment of Cardiac Arrhythmia

Guido Caluori, Annabelle Collin, Clair Poignard, Pierre Jais

Abstract Pulsed field ablation is a recent addition to the techniques used to ablate pathological arrhythmic circuits. Despite its recent introduction in the clinical field, pulsed field ablation exploits the well-known phenomenon of irreversible electroporation and the less known phenomena of selective cell death.

Far from being only used in cardiology, irreversible electroporation is mainly exploited in the treatment of solid tumors. Nevertheless, the tissue specificity of the technique with respect to muscular cells presented such a favourable benefits-to-risk ratio that fundamental questions (e.g., What causes cell death? Can we model it to predict the ablation lesion?) were put on hold in favour of preclinical testing and first clinical applications.

In this chapter, we explore the current knowledge of irreversible electroporation mechanisms and their modeling, keeping our focus on the research that put the basis and is setting the roadmap for pulsed field ablation in its promise to revolutionize the interventional treatment of arrhythmia.

1 Introduction

Endovascular catheter ablation of arrhythmia is a cornerstone of modern-day cardiac electrophysiology. Across 30 years of development and refinement it has allowed the treatment of both supraventricular and ventricular tachycardias, producing several ablation protocols, notably against atrial fibrillation as the most common one [36, 4]. Arrhythmic conditions represent a severe burden for patients' quality of life [52, 13]

Guido Caluori and Pierre Jais

IHU Liryc, INSERM U1045 and Fondation Bordeaux Universite, Avenue du Haut-Leveque 33600 Pessac, France, e-mail: guido.caluori@ihu-liryc.fr

Annabelle Collin and Clair Poignard

Institute of Mathematics of Bordeaux, University of Bordeaux 351 Cours de la Liberation 33405 Talence, France, e-mail: clair.poignard@inria.fr

and healthcare systems [43]. Atrial fibrillation alone, while not being lethal *per se*, imposes a strict anticoagulant and rate-control drug regime to prevent the occurrence of stroke or heart failure. Ventricular tachycardia, on the other hand, can require device implantation to prevent lethal prolonged tachycardia episodes deranging in ventricular fibrillation and sudden cardiac death.

Conventional ablation transfers thermal energy between a metal electrode and the endocardial surface of interest, to locally necrotize the tissue and break the abnormal electrical conduction circuits which cause the arrhythmia. Thermal energy can be transferred by increasing the temperature at the electrode-tissue interface via Joule heating, usually by applying a radiofrequency current between the endocardial electrode and an external reference patch. This approach, while proven superior to pharmacotherapy and highly versatile, suffers from different drawbacks (graphically represented in Fig. 1):

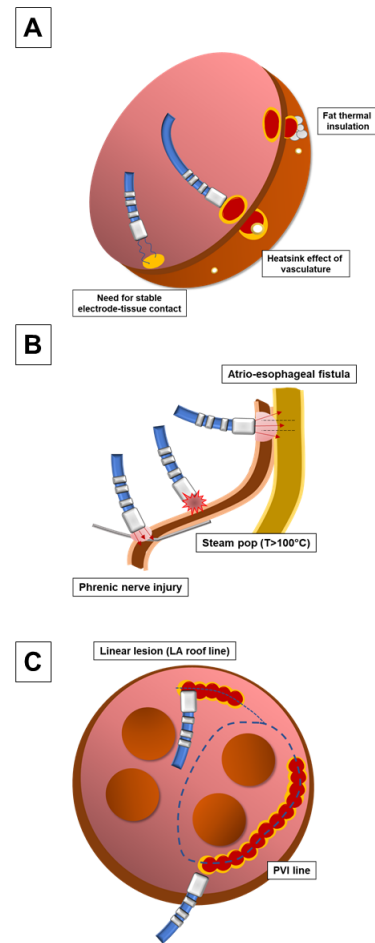


Fig. 1 Relevant issues with thermal-based ablation of cardiac arrhythmia (radiofrequency ablation is considered as reference): A) Heat sink effect from neighboring vasculature impairs the uniform growth of a thermal lesion; B) Thermal lesions can create steam-pops and grow to affect adjacent structures with possibly lethal damages (e.g. atrio-oesophageal fistula) C) Point-by-point ablation is required to achieve linear or complex lesion shapes, increasing the procedure time.

- temperature changes are contact-dependent, large vessels can function as heat sinks, and fat pads as insulators, thus impairing ablation transmuralit;
- thermal ablation is tissue-aspecific, therefore the risk of damage to multiple heart-adjacent structures is present (e.g., phrenic nerve, oesophagus, pleura), with potentially lethal consequences (i.e., atrio-oesophageal fistula);
- thermal ablation is usually performed point-by-point to maximize the precision of energy transfer, requiring long procedural times.

In order to reduce procedural time, for instance in case of pulmonary vein isolation (PVI) against paroxysmal atrial fibrillation, cryoballoon ablation has been introduced, where necrosis is quickly produced by temperatures in the order of -50°C . Despite similar efficacy as conventional radiofrequency ablation, this technique is mainly limited to PVI. Recent optimization of the combination of time and power for energy delivery, referred to as high-power-short-duration radiofrequency ablation, has provided partial improvement to the issue of procedure time. Nevertheless, none of these attempts do fully address the other and more significant issues related to thermal ablation.

Indeed, the ideal energy and approach to safely and effectively ablate arrhythmia would require the following properties:

- they must be tissue-specific, i.e., the physician must be able to target only the myocardium;
- they must be versatile and readily implementable with the catheter technology and protocols available;
- they must be fast to deploy and reliable in terms of lesion transmuralit and durability.

Historically, the first attempt to ablate arrhythmic circuits saw the use of DC current in the 1980s to create plasma membrane pores, which eventually killed cells by prolonged electroporation. Nevertheless, the delivery of high energy exposed the patients to severe risks of barotrauma and arcing and was soon substituted by RF ablation in the 1990s. Recent technologies can significantly reduce the duration of the DC energy pulse, as well as better control the shape of high-power ones. Indeed, the use of high-power-short-duration pulses has proven effective to treat solid tumors, and has been extensively studied for cardiac application. In this chapter we will summarize the use of electroporation for cardiac ablation purposes til the clinical introduction as pulsed field ablation (PFA). We will then explore mathematical and *in silico* approaches to better understand and plan PFA therapies. Ultimately we outline open issues that we feel need to be addressed in terms of fundamental and applied knowledge for the safe and effective spreading of PFA and its potentially paradigm-shifting benefits in cardiac electrophysiology.

Table 1 List of parameters and definitions relevant for electroporation and PFA

Parameter name	Unit	Definition
Amplitude	V ^a	Peak voltage imposed between electrodes (can be positive or negative)
Pulse width	ms - μ s	Duration of a single high-voltage stimulus
Interphase gap	ms - μ s	Time between the positive and negative phase of a biphasic stimulus
Pulse period	s - μ s	Duration of a single stimulus
Burst length ^b	s - μ s	Duration of the sequential pulse periods
Interburst interval	s - ms	Time between two pulse trains

^a Usually coupled with data on current (in A) ^b Also referred to as “application”

2 From irreversible electroporation to pulsed field ablation

Electroporation is a complex biological phenomenon in which the application of high intensity electric fields induces the formation of hydrophilic pores across the plasma membrane of living organisms. The electric field can be applied in several different ways, but always in the form of a single pulse or a sequence of identical pulses packed in a burst. Due to the highly heterogeneous use of pulse shapes and confusion in the parametric definition, we provide a short list (Table 1) and a graphic representation (Fig. 2) of the parameters of interest and their definition throughout this chapter for the readers’ reference.

The initial observations of field-induced defects in the cell membranes were followed by extensive application in cellular biology, mostly for the introduction of non-permeant molecules (e.g., drugs or plasmid DNA) into temporarily permeabilized cells *in vitro*. It was observed that when a sufficient level of electrical energy is supplied to the plasma membrane, the nanod defect density and stability are elevated so that their closure is energetically impaired. Thus, the cell membrane permeability changes are described as long-lasting, permanent, or irreversible, as observed *in vitro* and *in vivo*. There was evidence that secondary biochemical events caused by these defects (e.g., exhaustion of adenosine triphosphate, osmotic and electrolytic imbalance) can be potentially more damaging than membrane defects themselves. Remarkably, the major electroporation-induced damages occur within minutes or hours after the pulse application. This suggests that non-thermal irreversible electroporation (IRE) is the trigger of apoptotic processes at the cellular level. Together with the heterogeneous use and reporting of electroporative pulses, the cellular studies on cardiomyocytes are surprisingly limited, thus complicating the cell-specific translation of the results into clinical trials.

In the following sections, we provide an overview of the available preclinical knowledge on the phenomenon of cardiac IRE and its application in PFA, as well as clinical reports thereof.

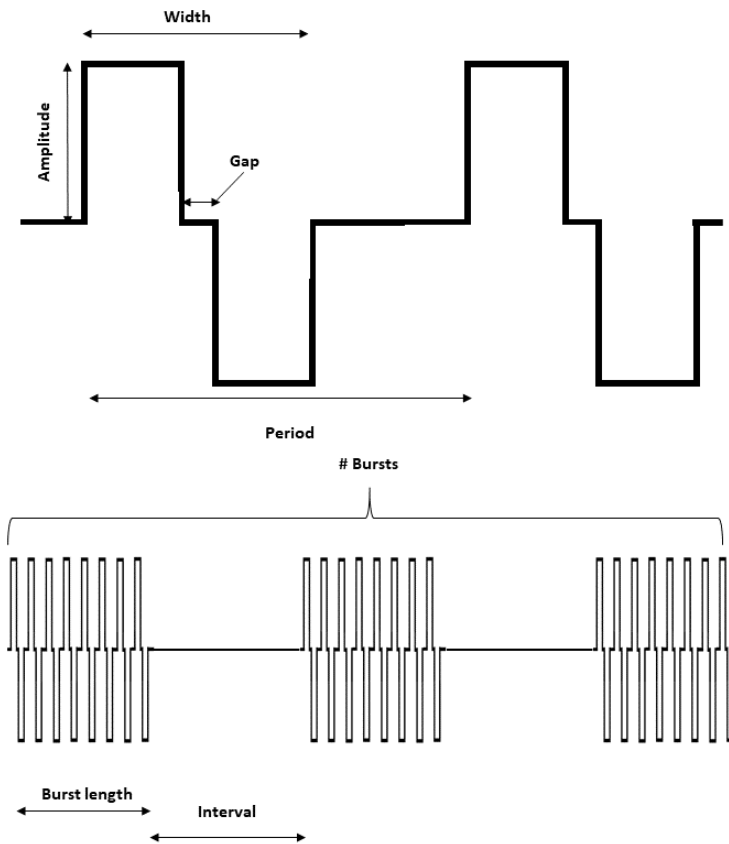


Fig. 2 PFA parameters and their definition.

2.1 Preclinical investigation

The cellular and *in vitro* characterization of IRE is challenging, mostly due to the difficulty and laboriousness in isolating adult cardiomyocytes, particularly from large animals. Therefore, most of the available literature deals with IRE evaluation on

cardiac cell lines. A unique case is represented by the first study on rat ventricular cardiomyocytes in 2008 by Xavier de Oliveira et al. Although their aim was to study how to prevent deleterious effects of cardioversion via IRE, they reported the first IC50 for cell death (50 V/cm). Moreover, this is the first report that calcium is necessary for the mechanism of irreversible cardiac injury upon electroporation [34]. This does not exclude other mechanisms possibly involved in cellular death. Feng et al. demonstrated that upon IRE in suspensions of HL-1, a significant amount of proteins is either lost (probably diffusing through the membrane pores) or denatured [24].

The individuation of a cardiac-specific threshold for IRE is common to several cellular studies: Kaminska et al. showed how H9C2 cardiac myoblasts display a threshold for induced cell death (measured by MTT assay after 24 h) at 375 V/cm [19]. Avazzadeh et al. studied the sensitivity of different cell line suspensions to IRE protocols, and noted that the HL-1 atrial cell line has a higher sensitivity than fibroblast-like and neuron-like cell lines [1]. This is remarkable if we consider that cell lines in suspensions have roughly the same spheroidal shape, thus pointing at different mechanisms - other than geometrical features - that determine cardiomyocyte sensitivity to IRE.

Dermol-Černe et al. also reported how electroporation (and presumably IRE) is dependent on the cell orientation with respect to the electric field in H9C2 and AC16 cell lines: interestingly nanosecond pulses caused more pronounced electroporation of the cells with perpendicular orientation, whilst the effects of microsecond pulses are not affected by cell orientation, and longer micro- to millisecond pulses have more effect on cells with parallel orientation. These observations, although at the single cell level, are of great importance for tissue with high anisotropy, such as the myocardium [?]

A seminal functional 2D *in vitro* study was presented recently by Hunter et al. [15]. Comparing different monolayer structures, including one of neonatal rat ventricular cardiomyocytes, they observed that electrode-to-monolayer distance inversely affects cardiac cell death probability, which nevertheless display an increased cell-type sensitivity to a symmetrical square biphasic shocks with a fixed duration of 10 ms. Furthermore, functional and permanent conduction block occurred when 50% to 80% of the cells near the electrode were killed, thus giving an interesting evidence of the correlation between damage and long-lasting effects.

In a unique *ex vivo* study in Langendorff perfused rabbit hearts, Wang et al. studied the effect of defibrillation shocks to procure acute and chronic electroporation observed by PI staining, reporting the acute and recoverable effects of IRE 45 min after shock [46].

Despite these interesting cellular studies, most of the preclinical studies were and are carried out *in vivo*. The first substantial set of works addressing cardiac ablation via PFA has been produced since 2012 by the team of Fred Wittkampf. A peculiarity of the work by this team and its later members is the use of monophasic exponential pulses delivered by a Lifepak 9 defibrillator (Physio-Control Inc., Redmond, WA, USA) against a reference patch, similarly to radiofrequency ablation. Consistently, they reported their single-pulse applications mainly in Joules, the unit displayed by the device. In their first open-chest porcine *in vivo* study, they tested two circular

custom-made catheters to deliver -10-200 J shock on the epicardium [48]. They report smooth, carbonization-free pulses with voltage ranges between 950 and 2150 V and a peak current between 15 and 37 A at a pulse duration of <10 ms. The lesion size increased proportionally to the energy delivered, with multi-polar catheters fusing their individual effects at higher energies. This first paper is of key importance because it highlighted already some key issues with PFA, notably that **tissue-specific regional differences and/or reference position might create preferential current paths that affect lesion formation** (deducted by reporting on unanticipated shape, in terms of width and depth, of few lesions).

Unanticipated lesion shapes in the ventricle were also reported by the group of Zdenek Starek at FNUSA-ICRC, where they used a set of biphasic ECG-triggered bursts and reported the formation of “doughnut-shaped” lesions across the interventricular septum, with a core of surviving cardiomyocytes (Fig. 3) [2].

Koruth et al. recently reiterated the difficulty in creating transmural lesions with biphasic pulses: they used the Farapulse Inc. (Menlo Park, CA, USA) bipolar catheter and delivery protocol targeted to the left and right ventricle in 4 endovascular porcine models. The compact and homogeneous lesions produced had an average depth of 6.5 ± 1.7 mm, insufficient to ablate transmurally across the whole ventricle [22]. Yavin et al. reported how the chronic lesions from the use of PFA in the ventricle - specifically using the Sphera9 lattice catheter (Affera Inc.,) - change significantly between single and multiple (i.e., four) applications at the same locations. From their results, it appears that with the tested PFA protocols they were unable to achieve depths typical of the ones achievable with the same catheter and RFA, thus suggesting the need for PFA optimization [51]. All groups reported no occurrence of ventricular arrhythmia during delivery.

Wittkampf’s group is not the only one to dedicate substantial effort to develop specialized catheters: the group led by Samuel Asirvatham at the Mayo Clinic

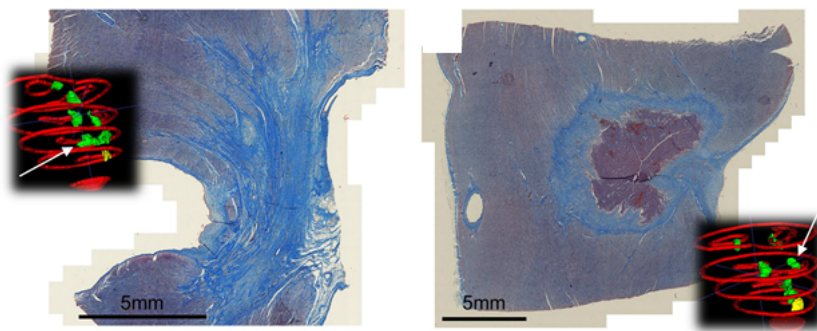


Fig. 3 Expected and unexpected PFA lesions across the interventricular septum, observed by Masson’s trichrome staining. On the left, a continuous lesion with wall shrinking localized as shown on the inset showing a 3D rendering of the heart. On the right, a more basal lesion containing a surviving core or myocardium, encircled by a “doughnut-shaped” fibrotic area. Adapted with permission from [2] under the Creative Commons Attribution 4.0 International License.

has proposed for instance a multipolar balloon catheter for the circular delivery of monophasic electroporative pulses, with included the drug-eluting possibility and appropriate acute safety [6]. Stewart et al. successfully tested the PVAC Gold circular catheter and ECG-gated delivery system by Medtronic for bipolar PFA delivery and showed the formation of transmural atrial lesions [42]. A subsequent study on energy titration showed that low-dose (± 700 V) pulses are also effective in the atria to produce durable lesions, at least at the right atrial location identified [41]. Yavin et al. describe the safety and feasibility of the Affera lattice catheter, which was designed to make focal lesion similarly to the ones produced by RFA [50], as well as the use of a new Biosense-Webster "LASSO" catheter (VARIPULSE) [49], with similar targets, endpoints and reported efficacy to create lesions. We mention more commercial systems with their dedicated technology in the next paragraph. Despite the research for a dedicated technology, Caluori et al. reported that commercially available diagnostic and ablation catheters can reliably produce transmural lesions with biphasic burst trains, although they did not verify compatibility of their PFA strategy with current electrophysiological recording systems and mapping during delivery (catheters and their sensors were without charring and functional after all applications) [2].

Neven et al. tested the possibility of performing epicardial linear lesions with a custom device, where the contact between electrode and tissue was assured by a suction cup. They reported successful lesion formation with a single application of 30-100-300 J, with proportionally increasing lesion dimensions and wall shrinking at higher energy, but no coronary intraluminal damage [28]. This finding is a key proof that PFA induced by IRE is tissue-specific and safer than thermal ablation. The team further assessed the feasibility and safety of their method for closed-chest epicardial ablation [29, 30]. Safety and specificity issues have been further addressed by performing PFA directly against several vulnerable regions:

- Pulmonary veins, where van Driel et al. observed that 200 J PFA shocks might lead to initial lumen diameter decrease ($11 \pm 10\%$ directly after ablation) but at 3 months, the lumen diameter was increased compatibly with animal growth. This behavior can be due to reversible vascular spasm and does not lead to chronic stenosis as shown in the RF control group [7]. These findings are also confirmed by work in a chronic canine model by Witt et al., where they reported transmural intraluminal lesions after monophasic PFA delivery, without stenosis (although with partial nerve damage at the location of the ganglionated plexus / intrinsic cardiac autonomic nervous system) [47]. The evidence of reduced stenosis upon PFA is reported also by Howard et al., who saw no damage to the lung structures with PFA [14].
- Phrenic nerve, where van Driel et al. reported that a 200 J shock at the location of the nerve - identified by endovascular capture in the superior caval vein - creates transmural circular lesions and does not affect the short-term and long-term functionality of the nerve [8]. Interestingly, Padmanabhan et al. tested a percutaneous epicardial ablation of ganglionated plexi, showing successful nerve alteration after 4 months without adipose or myocardial involvement [35]. **This**

last study secondarily shows how fat pads and infiltrates can shield the sensitive myocardium from PFA, thus possibly preventing optimal delivery.

- Esophagus, where shock delivery directly above the adventitia led to transient formation of intraepithelial vesicles (quickly disappearing) and small scarring in the outer layer of the muscular layer, generally preserving the tissue architecture [32].

Despite these remarkable contributions, monophasic pulses are known to lead to gas formation through electrolysis, a phenomenon which could cause gaseous microemboli in the systemic circulation and especially cause stroke in the brain. Biphasic pulses do not seem to be affected by this issue, as demonstrated by Caluori et al. by intracardiac echocardiography [2]. However, van Es et al. tested the formation of gaseous byproducts after monophasic shocks, noticing that gas formation is proportional to energy delivered, and asymmetric between cathode and anode, with cathodal (i.e., negative phase on the applicator with respect to the isoline) [9]. This was confirmed *in vivo* in an endocardial porcine model of atrial delivery, with remarkable gas production increase for cathodal pulses ($0.6 \pm 0.6 \mu\text{L}$ for anodal and $56.9 \pm 19.1 \mu\text{L}$ for cathodal applications, measured by a femoral bubble counter) [12]. In dogs, which have a similar cerebral vasculature as humans, they did not observe cerebral infarction by either MRI or histopathology after multiple 200 J anodal applications [33]. While this suggests that monophasic millisecond PFA is safe for the brain, it puts emphasis on the **need to carefully characterize each pulse shape and specific application for systemic safety**. Most recently, Neven et al. described the acute vs. chronic changes in seven pigs ablated with the custom linear electrode previously described. In their histological examination of lesions obtained in the first hour after shock delivery, they describe transmural oedema accompanied by a necrotic hypercontracture band in cardiomyocytes with eosinophilic infiltrates (the area is later substituted by connective tissue consistent with replacement fibrosis) [31]. The authors claim that PFA effects can be clinically tested without the waiting periods typically needed for thermal ablation.

2.2 Clinical investigation

Pulmonary vein isolation (PVI) to treat paroxysmal atrial fibrillation has been the first target of PFA clinical validation. Reddy et al. were the first in 2018 to report the safety and efficacy of PFA delivery with Farapulse protocols (then “Iowa Approach”) performed in 22 patients with both endovascular and surgical catheters. The quasi-total acute efficacy was remarkable when considering the total intervention time, limited to 67 ± 10.5 min [39]. The Farapulse PFA protocol, as it is deducible from the literature, is a single biphasic burst of ca. 500 ms in length (pulse width and period unknown) applied bipolarly across the splines of the Faraware catheter designed for PVI. A maximum amplitude of 2 kV of the generator imposes an electric field in the tissue of 4 kV/cm, roughly ten times higher than the reported IRE threshold for cardiomyocytes. Multiple applications at the same site were performed by rotating the catheter to ensure good contact on the whole circular site of ablation. Three clinical

trials have been carried on and reported so far with this technology, the IMPULSE, PEFCAT, and PEFCAT II, of which Reddy et al. provide a comprehensive summary in a recent work [38]. The main results are symbolic of the promise of PFA for the treatment of arrhythmia and are summarized below and in Fig. 4:

- Among the 121 patients enrolled and followed-up, acute PVI was achieved in 100% of them (7.2 ± 2.2 ablation lesions per PV and 28.1 ± 8.5 lesions per patient).
- The listed safety endpoints were reached in 2.5% of the treated patients (pericardial effusions probably due to aggressive catheter manipulation and vascular hematoma).
- The shape of the pulse and the optimization of the application led to a durable isolation, observed in remapping after 3 months: 96.0% of PVs ($n = 166$ of 173) and 84.1% of patients ($n = 37$ of 44). With the initial protocol 45.2% of PVs were isolated and all PVs in 18.1% of patients, stressing how dramatic the choice of the pulse features is.
- The survival analysis at 1 year follow-up showed freedom from AF was $81.1 \pm 3.8\%$ and freedom from AF/AFL (atrial flutter) or AT (atrial tachycardia) was $78.5 \pm 3.8\%$ in the overall cohort.
- The total area affected by PFA for PVI is comparable with the one affected by thermal methods [20].
- The area and the level of PV antral isolation with PFA does not seem to reverse over time, offering reassurance for the durable treatment of paroxysmal AF [21]. Nevertheless, this might not be true all across the heart, since it has been shown that - although using a different catheter - there is a degree of reversibility when targeting the atrial posterior wall to treat persistent AF, stressing again the possible existence of regional levels of myocardial susceptibility to PFA (PersAFOne trial [38]).

Of course the Farapulse system is not the only technology and protocol currently under validation in the open market for PFA-based therapies. Verma et al. reported on the first PVI in-human trial (PULSED AF) with the PulseSelect catheter and system for PFA produced by Medtronic, a direct evolution of the PVAC system. The protocol, in this case, consists of 4 bipolar bursts of 100-200 ms after a low-pulse check for phrenic nerve capture during a single cardiac cycle. Several applications per vein are repeated by rotating the catheter. Acute isolation was achieved in 100% of the 38 patients, with no side effects at 30 days related to PFA and temperature increases at the electrode level in the order of 2 [45].

Another interesting system, mostly for its ability to toggle between RFA and PFA, is the aforementioned Affera system, which uses a mapable spherical lattice catheter (Sphera9), which is specifically designed to deliver focal lesions. In a complex reporting of their first *in human* trial, the authors report on the combined use of RFA+PFA or solely PFA to achieve acute PVI and conduction block without adverse events in 76 patients. Acute electrical isolation was 100% effective in the 152 ipsilateral PV pairs targeted, with 19% requiring additional ablations. Mitral isthmus, cavotricuspid isthmus, and left atrium roof lines also successfully led to conduction block in 100%. One patient presented a femoral vascular hemorrhage,

while no patient presented oesophagus lesions with only PFA. Silent asymptomatic brain lesions were observed in 5.9% of the examined patients. The authors discuss, while stating that this rate is compatible with other studies and does not induce neurocognitive damages, that a better anticoagulative monitoring during the procedure can be beneficial [37].

Single-shot monophasic pulses have also been tested in clinical settings. In a small study of 10 patients, Loh et al. in 2020 showed that acutely all veins can be isolated by 2 applications of 200 J, 6 ms pulse via a custom non-deflectable 14-polar circular ablation catheter with a variable hoop diameter (16-27 mm). The peak voltage and current were reported as 2154 ± 59 V and 33.9 ± 1.6 A, and the multiple applications are considered to change position of the catheter and assure full circular contact with the tissue [25].

PFA presents several challenges for (extra)cardiac imaging and assessment of long-term ablation efficacy. For instance, Nakatani et al. [26] reported specific characteristics of PFA lesion progression during a single-center small chronic cardiac MRI study on patients affected by paroxysmal AF and treated with PFA (N = 18) or thermal ablation (N = 23, 16 with RFA and 7 with cryo-ablation). They observed that acutely (<3 h post-ablation), late-gadolinium enhancement (LGE) volume was 60% larger after PFA compared to thermal ablation ($p < 0.001$), and oedema on T2 imaging was 20% smaller ($p = 0.002$). Tissue changes were more homogeneous after PFA than after thermal ablation, with no sign of microvascular damage or intramural hemorrhage. In the chronic stage (3 months post-ablation), the majority of acute LGE had disappeared after PFA, whereas most LGE persisted after thermal ablation. The maximum strain on PV antra, the left atrium expansion index, and

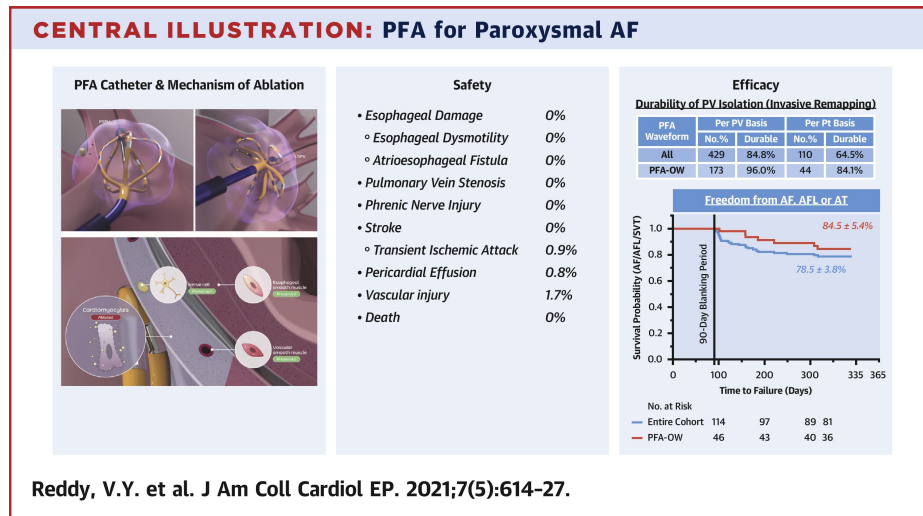


Fig. 4 Summary of the results from the IMPULSE, PEFCAT I/II trials, indicating the putative mechanism, safety and therapeutic outcomes.

left atrium active emptying fraction declined acutely after both PFA and thermal ablation but recovered at the chronic stage only with PFA. Imaging via LGE also allowed assessing the differential damages to the oesophagus and the aorta in a small study of 41 patients, where PFA showed its ability to spare the oesophageal structures (compared visible changes in 43% of patients with thermal methods), and interestingly a transitory change of the aortic wall (comparable with thermal method changes) of still unknown mechanism [3]. This study in particular reminds clinicians of the extra-cardiac effect of PFA, despite the overwhelming changes observed in the myocardium.

3 Modeling electroporation to aid clinical therapy

Modeling the mechanisms and the effects of high-voltage shocks can be of enormous assistance to PFA design and evaluation. Nevertheless, the possibilities in this field are still quite limited. The electroporation modeling of non-excitabile tissues is most of the time described by the following nonlinear electrostatic model [40, 16, 27, 44]. The tissue is described as a conductive medium, whose conductivity σ depends on the amplitude of the electric field $\mathbf{E} = -\nabla V$. The model reads then

$$-\nabla \cdot (\sigma(\|\nabla V\|)\nabla V) = 0, \quad \text{in } \Omega, \quad (1a)$$

$$V|_{\mathcal{E}^\pm} = g^\pm, \quad \text{on the active electrodes}, \quad (1b)$$

where \mathcal{E}^\pm denotes respectively the activated anode and cathode needles used for the pulse delivery, $\partial\Omega$ is the boundary of the domain Ω deprived of the activated needles, and g^\pm are the Dirichlet data (applied voltages) at the needle \mathcal{E}^\pm . Different conditions at the boundary of the domain Ω can be used (Neumann conditions if the domain is isolated, Dirichlet if the domain is set to the ground or Fourier-Robin to enable the electric flux to go out of the domain of computation). For small domains of computation (for instance to reduce the computational costs), the choice of the boundary condition is crucial and impacts the computation of the electric field markedly and thus requires particular attention. The classical description of tissue conductivity consists of a 4-parameter sigmoid function¹ describing phenomenologically the increase of electrical conductivity due to the magnitude of the electric field:

$$\forall \lambda \geq 0, \quad \sigma(\lambda) = \sigma_0 + \frac{\sigma_1 - \sigma_0}{2} (1 + ((\lambda - \mathbf{E}_{\text{th}}))), \quad (2)$$

where σ_0 is the conductivity of the non-electroporated tissue, σ_1 is the tissue conductivity of the fully porated tissue, \mathbf{E}_{th} is the threshold amplitude for electroporation, and λ is the slope of the nonlinearity.

¹ The choice of the sigmoid function does not seem essential according to Ivorra, Villedjeanne, Mir [17], likely because of the lack of experimental data. In the code we took a hyperbolic tangent but the function can be preferred.

This model has been extensively used in the context of tumor ablation by electroporation. For treatment planning purposes, the teams of Davalos and Miklavčič proposed different numerical procedures to optimize the needle positioning [5, 53, 11] to help clinicians determining the best localization of the needle. Poignard et al. used this model for procedural-dependent treated area computation [10, 23] to give a feedback on the procedure to clinicians. It is worth noting that other models have been developed but to our knowledge they are not used for clinical applications due to the high number of parameters. One can refer to the review by Jankowiack et al. [18] for a comparison of the existing models.

The model in equation (1) enables to provide a quite accurate description of the electric field distribution, at least for IRE purposes. Indeed, in IRE, the number of microsecond pulses is so high (several tens of bursts of about $100\mu\text{s}$ pair-to-pair) that it is likely that the static state is reached, justifying the use of equation (1). The typical information that can be obtained thanks to the modeling are the isolines of the electric field displayed on the medical images (see for instance Fig. 5) to assess the coverage of the tumor by the electric field.

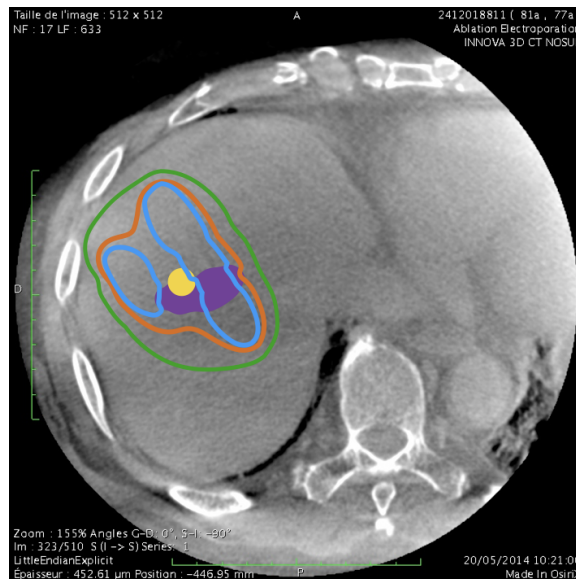


Fig. 5 Example of treatment assessment of hepatic tumor ablation by IRE. The electric field is computed with the electrostatic model (equation (1)) in the medical image (here the CBCT) framework. Isolines at 300 V/cm (green), 500 V/cm (red), and 750 V/cm (blue) are plotted on the CBCT. From Gallinato et al. [10].

4 Current open issues with pulsed field ablation and conclusions

With this overview, we can deduce several topics which are still source of debate and object of research, regarding both IRE and PFA.

- What causes the high susceptibility of cardiomyocytes to high-voltage shocks and cellular death induced by IRE? Different mechanisms are possibly involved, such as the relative orientation of the cell (which we saw in some conditions might present differences for elongated cells), its excitable nature (which determines ion fluxes across the membrane and potentially damaging calcium mishandling), and its contractile function (which makes it highly reliable on the tight balance between ATP production and consumption). Observing these phenomena is highly laborious and challenging, and we might still miss the important cell-by-cell interactions which might spread or limit the damage through gap junctions. While cells are essential, the use of more comprehensive systems, such as biomimetic slices, might be of better use to translate the results to PFA.
- How is PFA affected by tissue heterogeneity and procedural parameters? PFA works so far excellently in the atrial compartment, where the wall thickness and the tissue orientation is reduced compared to the ventricle. Nevertheless, our current knowledge already shows that transmural lesions in the ventricle cannot be readily produced with the PFA delivery protocols used for atrial fibrillation therapies. Whether this is simply due to tissue thickness or anisotropy, we do not know yet. Furthermore, we do not know how existing scarring and fibrofatty infiltration might affect the lesion formation with PFA even in the atria, an important issue for the ablation of more complex arrhythmia such as persistent atrial fibrillation - preclinical data suggest an unclear insulating role of adipose tissue, and fibrosis is not considered since the animals were all healthy models. Substantial work employing imaging and tissue characterization is still needed on this matter.
- PFA for cardiac tissue needs improvements on the modeling side. Indeed, the electrostatic model seems relevant for non-excitable tissues, but excitable tissues are usually described as a biphasic domain, where the extracellular and the intracellular phases play a major role in the transmembrane propagation. The corresponding model, the so-called bidomain model, thus involves two conductivities: intracellular and extracellular. It is not trivial how to adapt the electroporation model (equation (1)) to such biphasic models. It is worth noting that Voyer et al. proposed a biphasic model for electroporation that could be used in cardiology, but still the impact of the electropermeabilizing field on the ion channels has to be investigated. Another challenging issue is the post-pulse modeling of cardiac tissue. Indeed, these are composed of very different types of cells among them fibroblasts, which can proliferate and cardiomyocytes, which do not proliferate. Moreover, unlike radiofrequency ablation, PFA does not generate fibrosis, which can play a role in the relapse of fibrillation. In particular, from the numerical view point, it is not clear how to transmit the information from a non-affected region (where the bidomain model with the ion channel activity holds) with a

treated region, where most of the cardiomyocytes were killed. These transmission conditions are under investigation, and they could provide a first step in better understanding the PFA on cardiac tissue.

Finally, in the introduction we highlighted a few key ideal properties of an ablation energy and approach to treat cardiac arrhythmia. Based on the overview presented in this chapter, we can attempt to answer whether PFA, and electroporation at its base, fit these ideal properties:

- **It must be tissue-specific, i.e., the physician must be able to target only the myocardium:** PFA has proven to be remarkably tissue-selective for the myocardium, which has been the main appeal since its re-introduction in the clinical field.
- **It must be versatile and readily implementable with the catheter technology and protocols available:** despite the extensive ongoing commercial development of new catheters and dedicated systems, we believe certain PFA applications are already applicable with the existing catheter technology. High-voltage generators need an *ad hoc* design and the therapeutic protocols used to treat atrial arrhythmia so far could be smoothly translated from thermal ablation to PFA.
- **It must be fast to deploy and reliable in terms of lesion transmuralty and durability:** PFA lesions seem durable and the combination of catheter geometries and tissue sensitivity allow for a substantial reduction of intervention time compared to the existing standard.

In conclusion, we can hope - while carrying on with the necessary improvements and filling the fundamental knowledge gaps - that PFA will hold up to its promise to revolutionize the interventional treatment of cardiac arrhythmia.

Acknowledgements The authors acknowledge funding from the Atrial Fibrillation Chair of the IHU Liryc, Fondation Bordeaux Université, and the Fondation Lefoulon-Delalande. CP and AC are partly granted by the Plan Cancer project 21CM119-00 (MECI).

References

- [1] Avazzadeh S, O'Brien B, Coffey K, O'Halloran M, Keane D, Quinlan LR (????) Establishing irreversible electroporation electric field potential threshold in a suspension in vitro model for cardiac and neuronal cells. *Journal of Clinical Medicine* 10(22):5443, DOI 10.3390/jcm10225443
- [2] Caluori G, Odehnalova E, Jadczyk T, Pesl M, Pavlova I, Valikova L, Holzinger S, Novotna V, Rotrekl V, Hampl A, Crha M, Cervinka D, Starek Z (????) AC pulsed field ablation is feasible and safe in atrial and ventricular settings: A proof-of-concept chronic animal study. *Frontiers in Bioengineering and Biotechnology* 8
- [3] Cochet H, Nakatani Y, Sridi-Cheniti S, Cheniti G, Ramirez FD, Nakashima T, Eggert C, Schneider C, Viswanathan R, Derval N, Duchateau J, Pambrun T, Chauvel R, Reddy VY, Montaudon M, Laurent F, Sacher F, Hocini M,

- Haïssaguerre M, Jais P (????) Pulsed field ablation selectively spares the oesophagus during pulmonary vein isolation for atrial fibrillation. *EP Europace* 23(9):1391–1399, DOI 10.1093/europace/euab090
- [4] Cronin EM, Bogun FM, Maury P, Peichl P, Chen M, Namboodiri N, Aguinaga L, Leite LR, Al-Khatib SM, Anter E, Berruezo A, Callans DJ, Chung MK, Cuculich P, d'Avila A, Deal BJ, Della Bella P, Deneke T, Dickfeld TM, Hadid C, Haqqani HM, Kay GN, Latchamsetty R, Marchlinski F, Miller JM, Nogami A, Patel AR, Pathak RK, Sáenz Morales LC, Santangeli P, Sapp JL Jr, Sarkozy A, Soejima K, Stevenson WG, Tedrow UB, Tzou WS, Varma N, Zeppenfeld K, ESC Scientific Document Group (????) 2019 HRS/EHRA/APHRs/LAHRS expert consensus statement on catheter ablation of ventricular arrhythmias. *EP Europace* 21(8):1143–1144, DOI 10.1093/europace/euz132
- [5] Davalos RV, Bhonsle S, Neal II RE (????) Implications and considerations of thermal effects when applying irreversible electroporation tissue ablation therapy. *The Prostate* 75(10):1114–1118, DOI 10.1002/pros.22986
- [6] DeSimone CV, Ebrille E, Syed FF, Mikell SB, Suddendorf SH, Wahnschaffe D, Ladewig DJ, Gilles EJ, Danielsen AJ, Holmes DR, Asirvatham SJ (????) Novel balloon catheter device with pacing, ablating, electroporation, and drug-eluting capabilities for atrial fibrillation treatment—preliminary efficacy and safety studies in a canine model. *Translational Research* 164(6):508–514, DOI 10.1016/j.trsl.2014.07.002
- [7] van Driel VJ, Neven KG, van Wessel H, du Pré BC, Vink A, Doevendans PA, Wittkampf FH (????) Pulmonary vein stenosis after catheter ablation. *Circulation: Arrhythmia and Electrophysiology* 7(4):734–738, DOI 10.1161/CIRCEP.113.001111
- [8] van Driel VJHM, Neven K, van Wessel H, Vink A, Doevendans PAFM, Wittkampf FHM (????) Low vulnerability of the right phrenic nerve to electroporation ablation. *Heart Rhythm* 12(8):1838–1844, DOI 10.1016/j.hrthm.2015.05.012
- [9] van Es R, Groen MHA, Stehouwer M, Doevendans PA, Wittkampf FHM, Neven K (????) In vitro analysis of the origin and characteristics of gaseous microemboli during catheter electroporation ablation. *Journal of Cardiovascular Electrophysiology* 30(10):2071–2079, DOI 10.1111/jce.14091
- [10] Gallinato O, Senneville BDd, Seror O, Poinard C (????) Numerical workflow of irreversible electroporation for deep-seated tumor. *Physics in Medicine & Biology* 64(5):055,016, DOI 10.1088/1361-6560/ab00c4
- [11] Garcia PA, Kos B, Rossmeisl Jr JH, Pavliha D, Miklavčič D, Davalos RV (????) Predictive therapeutic planning for irreversible electroporation treatment of spontaneous malignant glioma. *Medical Physics* 44(9):4968–4980, DOI 10.1002/mp.12401
- [12] Groen MHA, van Es R, van Klarenbosch BR, Stehouwer M, Loh P, Doevendans PA, Wittkampf FH, Neven K (????) In vivo analysis of the origin and characteristics of gaseous microemboli during catheter-mediated irreversible electroporation. *EP Europace* 23(1):139–146, DOI 10.1093/europace/euaa243

- [13] Guandalini GS, Liang JJ, Marchlinski FE (????) Ventricular tachycardia ablation: Past, present, and future perspectives. *JACC: Clinical Electrophysiology* 5(12):1363–1383, DOI 10.1016/j.jacep.2019.09.015
- [14] Howard B, Haines DE, Verma A, Packer D, Kirchhof N, Barka N, Onal B, Fraasch S, Miklavčič D, Stewart MT (????) Reduction in pulmonary vein stenosis and collateral damage with pulsed field ablation compared with radiofrequency ablation in a canine model. *Circulation: Arrhythmia and Electrophysiology* 13(9):e008,337, DOI 10.1161/CIRCEP.120.008337
- [15] Hunter DW, Kostecki G, Fish JM, Jensen JA, Tandri H (????) In vitro cell selectivity of reversible and irreversible. *Circulation: Arrhythmia and Electrophysiology* 14(4):e008,817, DOI 10.1161/CIRCEP.120.008817
- [16] 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. *Physics in Medicine and Biology* 54(19):5949–5963, DOI 10.1088/0031-9155/54/19/019
- [17] Ivorra A, Villemejane J, Mir LM (????) Electrical modeling of the influence of medium conductivity on electroporation. *Physical Chemistry Chemical Physics* 12(34):10,055–10,064, DOI 10.1039/C004419A
- [18] Jankowiak G, Taing C, Poignard C, Collin A (????) Comparison and calibration of different electroporation models. application to rabbit livers experiments. *ESAIM: Proceedings and Surveys* 67:242–260, DOI 10.1051/proc/202067014
- [19] Kaminska I, Kotulska M, Stecka A, Saczko J, Drag-Zalesinska M, Wysocka T, Choromanska A, Skolucka N, Nowicki R, Marczak J, Kulbacka J (????) Electroporation-induced changes in normal immature rat myoblasts (h9c2). *General physiology and biophysics* 31(1):19–25, DOI 10.4149/gpb_2012_003
- [20] Kawamura I, Neuzil P, Shivamurthy P, Kuroki K, Lam J, Musikantow D, Chu E, Turagam MK, Minami K, Funasako M, Petru J, Choudry S, Miller MA, Langan MN, Whang W, Dukkipati SR, Koruth JS, Reddy VY (????) How does the level of pulmonary venous isolation compare between pulsed field ablation and thermal energy ablation (radiofrequency, cryo, or laser)? *EP Europace* 23(11):1757–1766, DOI 10.1093/europace/euab150
- [21] Kawamura I, Neuzil P, Shivamurthy P, Petru J, Funasako M, Minami K, Kuroki K, Dukkipati SR, Koruth JS, Reddy VY (????) Does pulsed field ablation regress over time? a quantitative temporal analysis of pulmonary vein isolation. *Heart Rhythm* 18(6):878–884, DOI 10.1016/j.hrthm.2021.02.020
- [22] Koruth JS, Kuroki K, Iwasawa J, Viswanathan R, Brose R, Buck ED, Donskoy E, Dukkipati SR, Reddy VY (????) Endocardial ventricular pulsed field ablation: a proof-of-concept preclinical evaluation. *Europace: European Pacing, Arrhythmias, and Cardiac Electrophysiology: Journal of the Working Groups on Cardiac Pacing, Arrhythmias, and Cardiac Cellular Electrophysiology of the European Society of Cardiology* 22(3):434–439, DOI 10.1093/europace/euz341
- [23] Lafitte L, Giraud R, Zachiu C, Ries M, Sutter O, Petit A, Seror O, Poignard C, de Senneville BD (????) Patch-based field-of-view matching in multi-modal

- images for electroporation-based ablations. *Computerized Medical Imaging and Graphics* 84:101,750, DOI 10.1016/j.compmedimag.2020.101750
- [24] Liu F, Roy P, Shao Q, Jiang C, Choi J, Chung C, Mehra D, Bischof JC (???) The role of protein loss and denaturation in determining outcomes of heating, cryotherapy, and irreversible electroporation on cardiomyocytes. *Journal of Biomechanical Engineering* 140(6), DOI 10.1115/1.4039375
- [25] Loh P, van Es R, Groen MH, Neven K, Kassenberg W, Wittkamp F, Doevendans PA (???) Pulmonary vein isolation with single pulse irreversible electroporation. *Circulation: Arrhythmia and Electrophysiology* 13(10):e008,192, DOI 10.1161/CIRCEP.119.008192
- [26] Nakatani Y, Sridi-Cheniti S, Cheniti G, Ramirez FD, Goujeau C, André C, Nakashima T, Eggert C, Schneider C, Viswanathan R, Krisai P, Takagi T, Kamakura T, Vlachos K, Derval N, Duchateau J, Pambrun T, Chauvel R, Reddy VY, Montaudon M, Laurent F, Sacher F, Hocini M, Haïssaguerre M, Jais P, Cochet H (???) Pulsed field ablation prevents chronic atrial fibrotic changes and restrictive mechanics after catheter ablation for atrial fibrillation. *EP Europace* 23(11):1767–1776, DOI 10.1093/europace/euab155
- [27] Neal RE, Rossmeisl JH, Garcia PA, Lanz OI, Henao-Guerrero N, Davalos RV (???) Successful treatment of a large soft tissue sarcoma with irreversible electroporation. *Journal of Clinical Oncology* 29(13):e372–e377, DOI 10.1200/JCO.2010.33.0902
- [28] Neven K, van Driel V, van Wessel H, van Es R, Doevendans PA, Wittkamp F (???) Epicardial linear electroporation ablation and lesion size. *Heart Rhythm* 11(8):1465–1470, DOI 10.1016/j.hrthm.2014.04.031
- [29] Neven K, van Driel V, van Wessel H, van Es R, Doevendans PA, Wittkamp F (???) Myocardial lesion size after epicardial electroporation catheter ablation after subxiphoid puncture. *Circulation: Arrhythmia and Electrophysiology* 7(4):728–733, DOI 10.1161/CIRCEP.114.001659
- [30] Neven K, van Driel V, van Wessel H, van Es R, du Pré B, Doevendans PA, Wittkamp F (???) Safety and feasibility of closed chest epicardial catheter ablation using electroporation. *Circulation: Arrhythmia and Electrophysiology* 7(5):913–919, DOI 10.1161/CIRCEP.114.001607
- [31] Neven K, van Driel VJHM, Vink A, du Pré BC, van Wessel H, Fütting A, Doevendans PA, Wittkamp FHM, van Es R (???) Characteristics and time course of acute and chronic myocardial lesion formation after electroporation ablation in the porcine model. *Journal of Cardiovascular Electrophysiology* 33(3):360–367, DOI 10.1111/jce.15352
- [32] Neven K, van Es R, van Driel V, van Wessel H, Fidler H, Vink A, Doevendans P, Wittkamp F (???) Acute and long-term effects of full-power electroporation ablation directly on the porcine esophagus. *Circulation: Arrhythmia and Electrophysiology* 10(5):e004,672, DOI 10.1161/CIRCEP.116.004672
- [33] Neven K, Fütting A, Byrd I, Heil RW, Fish JM, Feeney DA, Donskoy E, Jensen JA (???) Absence of (sub-)acute cerebral events or lesions after electroporation ablation in the left-sided canine heart. *Heart Rhythm* 18(6):1004–1011, DOI 10.1016/j.hrthm.2021.02.015

- [34] de Oliveira PX, Bassani RA, Bassani* JWM (????) Lethal effect of electric fields on isolated ventricular myocytes. *IEEE Transactions on Biomedical Engineering* 55(11):2635–2642, DOI 10.1109/TBME.2008.2001135
- [35] Padmanabhan D, Naksuk N, Killu AK, Kapa S, Witt C, Sugrue A, DeSimone CV, Madhavan M, de Groot JR, O'Brien B, Rabbette T, Coffey K, Asirvatham SJ (????) Electroporation of epicardial autonomic ganglia: Safety and efficacy in medium-term canine models. *Journal of Cardiovascular Electrophysiology* 30(4):607–615, DOI 10.1111/jce.13860
- [36] Parameswaran R, Al-Kaisey AM, Kalman JM (????) Catheter ablation for atrial fibrillation: current indications and evolving technologies. *Nature Reviews Cardiology* 18(3):210–225, DOI 10.1038/s41569-020-00451-x
- [37] Reddy VY, Anter E, Rackauskas G, Peichl P, Koruth JS, Petru J, Funasako M, Minami K, Natale A, Jais P, Nakagawa H, Marinskis G, Aidietis A, Kautzner J, Neuzil P (????) Lattice-tip focal ablation catheter that toggles between radiofrequency and pulsed field energy to treat atrial fibrillation. *Circulation: Arrhythmia and Electrophysiology* 13(6):e008,718, DOI 10.1161/CIRCEP.120.008718
- [38] Reddy VY, Dukkipati SR, Neuzil P, Anic A, Petru J, Funasako M, Cochet H, Minami K, Breskovic T, Sikiric I, Sediva L, Chovanec M, Koruth J, Jais P (????) Pulsed field ablation of paroxysmal atrial fibrillation: 1-year outcomes of IMPULSE, PEFCAT, and PEFCAT II. *JACC: Clinical Electrophysiology* 7(5):614–627, DOI 10.1016/j.jacep.2021.02.014
- [39] Reddy VY, Koruth J, Jais P, Petru J, Timko F, Skalsky I, Hebel R, Labrousse L, Barandon L, Kralovec S, Funosako M, Mannuva BB, Sediva L, Neuzil P (????) Ablation of atrial fibrillation with pulsed electric fields: An ultra-rapid, tissue-selective modality for cardiac ablation. *JACC: Clinical Electrophysiology* 4(8):987–995, DOI 10.1016/j.jacep.2018.04.005
- [40] Sel D, Cukjati D, Batuskaite D, Slivnik T, Mir L, Miklavcic D (????) Sequential finite element model of tissue electropermeabilization. *IEEE Transactions on Biomedical Engineering* 52(5):816–827, DOI 10.1109/TBME.2005.845212
- [41] Stewart MT, Haines DE, Miklavčič D, Kos B, Kirchhof N, Barka N, Mattison L, Martien M, Onal B, Howard B, Verma A (????) Safety and chronic lesion characterization of pulsed field ablation in a porcine model. *Journal of Cardiovascular Electrophysiology* 32(4):958–969, DOI 10.1111/jce.14980
- [42] Stewart MT, Haines DE, Verma A, Kirchhof N, Barka N, Grassl E, Howard B (????) Intracardiac pulsed field ablation: Proof of feasibility in a chronic porcine model. *Heart Rhythm* 16(5):754–764, DOI 10.1016/j.hrthm.2018.10.030
- [43] Tang DH, Gilligan AM, Romero K (????) Economic burden and disparities in healthcare resource use among adult patients with cardiac arrhythmia. *Applied Health Economics and Health Policy* 12(1):59–71, DOI 10.1007/s40258-013-0070-9
- [44] Thomson KR, Kavnoudias H, Neal RE (????) Introduction to irreversible electroporation—principles and techniques. *Techniques in Vascular & Interventional Radiology* 18(3):128–134, DOI 10.1053/j.tvir.2015.06.002
- [45] Verma A, Boersma L, Haines DE, Natale A, Marchlinski FE, Sanders P, Calkins H, Packer DL, Hummel J, Onal B, Rosen S, Kuck KH, Hindricks G, Wilsmore B

- (???) First-in-human experience and acute procedural outcomes using a novel pulsed field ablation system: The PULSED AF pilot trial. *Circulation: Arrhythmia and Electrophysiology* 15(1):e010,168, DOI 10.1161/CIRCEP.121.010168
- [46] Wang YT, Efimov IR, Cheng Y (???) Electroporation induced by internal defibrillation shock with and without recovery in intact rabbit hearts. *American Journal of Physiology-Heart and Circulatory Physiology* 303(4):H439–H449, DOI 10.1152/ajpheart.01121.2011
- [47] Witt CM, Sugrue A, Padmanabhan D, Vaidya V, Gruba S, Rohl J, DeSimone CV, Killu AM, Naksuk N, Pederson J, Suddendorf S, Ladewig DJ, Maor E, Holmes DR, Kapa S, Asirvatham SJ (???) Intrapulmonary vein ablation without stenosis: A novel balloon-based direct current electroporation approach. *Journal of the American Heart Association* 7(14):e009,575, DOI 10.1161/JAHA.118.009575
- [48] Wittkamp FH, van Driel VJ, van Wessel H, Neven KG, Gründeman PF, Vink A, Loh P, Doevendans PA (???) Myocardial lesion depth with circular electroporation ablation. *Circulation: Arrhythmia and Electrophysiology* 5(3):581–586, DOI 10.1161/CIRCEP.111.970079
- [49] Yavin H, Brem E, Zilberman I, Shapira-Daniels A, Datta K, Govari A, Altmann A, Anic A, Wazni O, Anter E (???) Circular multielectrode pulsed field ablation catheter lasso pulsed field ablation. *Circulation: Arrhythmia and Electrophysiology* 14(2):e009,229, DOI 10.1161/CIRCEP.120.009229
- [50] Yavin H, Shapira-Daniels A, Barkagan M, Sroubek J, Shim D, Melidone R, Anter E (???) Pulsed field ablation using a lattice electrode for focal energy delivery. *Circulation: Arrhythmia and Electrophysiology* 13(6):e008,580, DOI 10.1161/CIRCEP.120.008580
- [51] Yavin HD, Higuchi K, Sroubek J, Younis A, Zilberman I, Anter E (???) Pulsed-field ablation in ventricular myocardium using a focal catheter. *Circulation: Arrhythmia and Electrophysiology* 14(9):e010,375, DOI 10.1161/CIRCEP.121.010375
- [52] Zhang L, Gallagher R, Neubeck L (???) Health-related quality of life in atrial fibrillation patients over 65 years: A review. *European Journal of Preventive Cardiology* 22(8):987–1002, DOI 10.1177/2047487314538855
- [53] Zupanic A, Kos B, Miklavcic D (???) Treatment planning of electroporation-based medical interventions: electrochemotherapy, gene electrotransfer and irreversible electroporation. *Physics in Medicine and Biology* 57(17):5425–5440, DOI 10.1088/0031-9155/57/17/5425