Arrhythmia-Related Cardiovascular Disease

Subjects: Cardiac & Cardiovascular Systems

Contributor: Leo Quinlan

Targeted cellular ablation is being increasingly used in the treatment of arrhythmias and structural heart disease. Catheter-based ablation for atrial fibrillation (AF) is considered a safe and effective approach for patients who are medication refractory. Electroporation (EPo) employs electrical energy to disrupt cell membranes which has a minimally thermal effect. The nanopores that arise from EPo can be temporary or permanent. Reversible electroporation is transitory in nature and cell viability is maintained, whereas irreversible electroporation causes permanent pore formation, leading to loss of cellular homeostasis and cell death. Several studies report that EPo displays a degree of specificity in terms of the lethal threshold required to induce cell death in different tissues. However, significantly more research is required to scope the profile of EPo thresholds for specific cell types within complex tissues. Irreversible electroporation (IRE) as an ablative approach appears to overcome the significant negative effects associated with thermal based techniques, particularly collateral damage to surrounding structures. With further fine-tuning of parameters and longer and larger clinical trials, EPo

Keywords: electroporation ; pulsed field ablation ; cardiac ; heart ; arrhythmia ; atrial fibrillation

1. Introduction

The Centers for Disease Control and Prevention in the USA reports that 1 in every 4 deaths in the United States is related to general cardiovascular disease, with an estimated 12.1 million people predicted to develop arrhythmias such as atrial fibrillation (AF) by 2030 [1]. In recent years there has been a rapid growth in the technology base and clinical appetite for targeted ablative procedures for arrhythmias, with some reports showing procedures to be effective, with quick procedural timelines, minimal associated risks and rapid recovery times [2][3]. Catheter-based ablation for AF is considered a safe and effective approach for patients who are refractory to medication. The cornerstone of catheter-based approaches to date is pulmonary vein isolation (PVI) but, increasingly, additional sites beyond the pulmonary veins are now being targeted [4]. In this review we report on the available data exploring energy-based ablative technologies, highlight the differing modalities that have been developed with a particular focus on anti-arrhythmic therapies. This review also considers the factors involved in achieving successful ablation of cardiac tissue and the evidence from in vitro and in vivo preclinical work which has informed clinical studies using EPo approaches.

2. Current Ablation Approaches for Treating Arrhythmia

Several relatively simple non-invasive ablative procedures have been developed to date, such as alcohol septal ablation, which involves the injection of ethanol into the septal coronary artery to target portions of the septal wall [5]. This minimally invasive ablation method has been extensively employed as a treatment for structural related heart defects such as hypertrophic cardiomyopathy, targeting the attenuation of outflow tract obstruction [2][6]. Alcohol septal ablation is often applied when previous lower intensity therapies have failed [5]. Stereotactic radioablation is another non-invasive modality under development. While not currently used in clinical practice to the best of our knowledge, a number of animal-based feasibility studies with stereotactic radioablation have been performed and reviewed elsewhere [7][8].

Typically, more invasive ablation techniques require entry into the body cavity to access targeted areas of the myocardium (Figure 1). These techniques up to more recently generally involved the use of thermal energy and either induced hyper- or hypo-thermal injury at the target site [9]. Hyperthermal approaches are most commonly based on the application of radiofrequency (RF) or laser energy. Hypothermal approaches, termed cryoablation, are commonly achieved by passing cooled, thermally conductive, fluids through hollow probes at the target site.

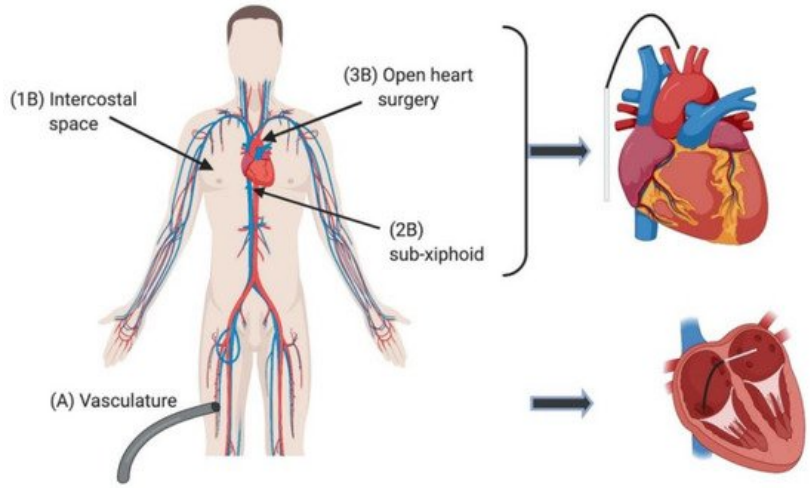

Figure 1. Access to the heart for invasive ablation purposes. This can be achieved via an internal endocardial approach (**A**) via the femoral vasculature (Table 1). Ablation catheter access can also be gained from an external epicardial (B) method. The extremities of the heart are reached by this technique. Access via an epicardial approach can be achieved through ports in the intercostal spaces (**1B**), a sub-xiphoid puncture (**2B**) or via open heart surgery (**3B**). The choice made between the two approaches is often made in relation to the target area and patient's disease substrate [10].

Table 1. Comparison of preclinical IRE studies on cardiac tissue.

Ref.	Subject	Follow-Up	Energy	Parameters	Monophasic/Biphasic Waveform	Monopolar/Bipolar Electrode Configuration	Reported Outcome
In Vitro							
[3]	HL-1 cell line	N/A	200 V; 1000 V/cm	PD- 50 µs, F- 10 Hz, PF- 10, 50, 99 pulses.	Not specified	Not specified	(1) IRE is effective for creating lesions on HL-1 cell line.
[11]	Cardiac strand-2D model	N/A	0.4–0.5 V; 25 V/cm	PD- 5 ms	Monophasic	Not specified	(1) Cardiac fibre exposed to a strong stimulus responds by developing pores in the first layer of cells immediately adjacent to the electrode. (2) IRE stops the growth of the macroscopic transmembrane potential, it does not affect intra- and extracellular potentials in the bulk of the tissue.
In Vivo Animal							

Ref.	Subject	Follow-Up	Energy	Parameters	Monophasic/Biphasic Waveform	Monopolar/Bipolar Electrode Configuration	Reported Outcome
[2]	Rat	1 month	50, 250, 500 V	PD- 70 vs. 100 µs, F- 1, 2, 3, 4 Hz, PF- 10 V's 20.	Not specified	Not specified	(1) Longer pulse duration (100 µs vs. 70 µs) is associated with larger volume reduction. (2) More pulses (20 vs. 10) are associated with larger volume reduction. (3) Pulse voltage (500 V vs. 250 V, 50 V) has an important effect on tissue damage. (4) Lower pulse frequency (10 Hz vs 20 Hz) is correlated with harsher tissue damage.
[9]	Porcine	24 h	1500–2000 V	PD- 100 µs, PF- 8, 16, 32.	Not specified	Not specified	(1) Lesions were mean 0.9 cm in depth. (2) Complete transmural destruction of atrial tissue at the site of the electrode application. (3) No local temperature change and with demonstration of electrical isolation.
[12]	Porcine	7 days	Not specified	F- 1 Hz, PF- 35	Not specified	Bipolar	(1) Unlike RF lesions, SW lesions showed only mild denaturation and little disruption of endocardium. (2) Lesion depth from SW correlated to amount of energy used. (3) SWCA lesions showed transient inflammatory responses followed by accelerated healing process with preserved myocardial blood flow.
[13]	Porcine	3 weeks	Not specified	Not specified	Monophasic	Not specified	(1) Mean depths ranged from 2.9 + 1.2 mm–6.5 + 2.7 mm. (2) 32% of lesions were transmural. (3) Coronary arteries do not develop significant stenosis within 3 weeks after epicardial IRE.

Ref.	Subject	Follow-Up	Energy	Parameters	Monophasic/Biphasic Waveform	Monopolar/Bipolar Electrode Configuration	Reported Outcome
[14]	Porcine	3 months	Not specified	PF- 3.	Monophasic	Not specified	(1) Mean value of the median lesion depths was 6.4 ± 2.6 mm. (2) 31% of lesions were transmural. (3) Apart from short-lasting (<30 min) coronary spasm, no long-term luminal narrowing was seen.
[15]	Porcine	2 weeks	500 V	PD- 90 µs, PF- 60.	Biphasic	Bipolar	(1) PFA lesions comparable to RFA lesions and had no collateral damage.
[16]	Canine	29 days	750 V	PD- 20 µs, F- 30–500 Hz, PF-10.	Not specified	Bipolar	(1) PEF can safely ablate Purkinje fibres. (2) Minimal collateral damage to myocardium.
[17]	Porcine	3 weeks	Not specified	PF- 4.	Monophasic	Bipolar	(1) Low energy IRE is safe and efficient in creating lesions on the PV ostia.
[18]	Rat	N/A	20 kV; 36 kV/cm	PD- 10 ns, F- 2 Hz, PF-3.	Not specified	Not specified	(1) nsEP produces smaller pore size and reduced non-polar distribution of electro-pores over the cell body. (2) At near threshold intensities, both nsEPo and msEPo triggered Ca^{2+} transients.
[19]	Rabbit	N/A	50–500 V	F- 1–2 kHz, PF- 6–10.	Monophasic	Bipolar	(1) IRE thresholds were 229 ± 81 and 318 ± 84 V for the endocardium and the epicardium, respectively. (2) Selective transient impairment of electrical activity in endocardial bundles is caused by IRE. (3) IRE might transiently reduce myocardial vulnerability to arrhythmias.
[20]	Ovine	N/A	Not specified	PD- 100–400 µs, F- 1–5 Hz, PF- 10–40 pulses.	Not specified	Bipolar	(1) Lesions were well demarcated from the unaffected tissue. (2) The induced inflammatory reaction within these acute ablations was minimal.

Ref.	Subject	Follow-Up	Energy	Parameters	Monophasic/Biphasic Waveform	Monopolar/Bipolar Electrode Configuration	Reported Outcome
[21]	Porcine	3 weeks	600 V	PD- 2 ms, F- 10 kHz, PF- 10.	Biphasic	Not specified	(1) Demonstrated the feasibility of a novel asymmetrical high frequency (aHF) waveform for IRE. (2) The aHF waveform led to significantly deeper lesions than the symmetrical HF waveform. (3) Both methods showed lesions of more than 4 mm deep.
[22]	Murine, rat, porcine	N/A	100 V; 12.2 kV/cm	PD- 400 ns, PF- 20.	Not specified	Not specified	(1) Stimulation by 200 ns shocks can elicit Ca^{2+} transients. (2) Shortest shocks cause the least damage and their threshold energy is minimal. (3) Orientation of cardiomyocytes with respect for electric field does not affect threshold for ns shocks.
[23]	Murine	N/A	Not specified	PD- 200 µs	Not specified	Not specified	(1) 200 ns stimuli induced action potentials. (2) nsPEF caused Ca^{2+} entry, associated with a slow sustained depolarisation.
[24]	Rabbit	N/A	200 V	PD- 350 ns, F- 1, 3 Hz, PF- 20, 6.	Not specified	Monopolar	(1) Nonconducting lesions created in less than 2 s with nsPEF application per site and minimal heating (<0.2 °C) of the tissue. (2) Lesion was smoother and more uniform throughout the wall in comparison to RF lesions.
[25]	Canine	113 ± 7 days	1000 V	PD- 100 µs, PF- 10	Not specified	Bipolar	(1) Cardiac GP permanently damaged using DC for IRE. (2) Preservation of atrial myocardial architecture and absence of inflammatory reaction and fibrosis.
[26]	Porcine	63 ± 3.3 days	800–1800 V	Not specified	Monophasic	Bipolar	(1) Both waveforms created confluent myocardial lesions. (2) Biphasic PFA was more durable than monophasic PFA and radiofrequency ablation lesions.

Ref.	Subject	Follow-Up	Energy	Parameters	Monophasic/Biphasic Waveform	Monopolar/Bipolar Electrode Configuration	Reported Outcome
[27]	Rabbit	4 weeks	300 V	Not specified	Monophasic	Bipolar	(1) Shock-induced IRE was spatially dependent on the location and dimension of the active region of the shock electrode. (2) The surviving anterior epicardial layers in the infarcted region were more susceptible to IRE.
[28]	Rabbit	Not specified	200 V; 3 kV/cm	PD- 350 ns, F- 3 Hz, PF- 6.	Not specified	Not specified	(1) High anisotropy ratio substantially affects the ablation outcome, low anisotropy ratio does not.
[29]	Porcine	3 months	Not specified	Not specified	Monophasic	Not specified	(1) Lesion size, depth and width corresponds to magnitude of energy used. (2) Initial spasm of coronary vasculature was noted, but this did not persist and was not recorded at follow-up.
[30]	Porcine	3 months	Not specified	Not specified	Not specified	Not specified	(1) Mean depth of the 30 J, 100 J and 300 J lesions was 3.2 ± 0.7, 6.3 ± 1.8 and 8.0 ± 1.5 mm, respectively. (2) Mean width of the 30 J, 100 J, and 300 J lesions was 10.1 ± 0.8, 15.1 ± 1.5 and 17.1 ± 1.3 mm, respectively. (3) No luminal arterial narrowing was observed after 3 months.
[31]	Porcine	3 weeks	950–2150 V	PD- <10 ms, PF- 4.	Monophasic	Monopolar	(1) 200 J applications yielded median lesion depth of 5.2 ± 1.2 mm. (2) No signs of tissue heating. (3) Lesion would be sufficient for inducing PVI.
[32]	Canine	N/A	Not specified	PD- 60–300 s, F- 7 kHz.	Not specified	Not specified	(1) Device can successfully deliver both RF and IRE energy. (2) Addition of porous configuration on balloon can aid in enhancing drug delivery.

Ref.	Subject	Follow-Up	Energy	Parameters	Monophasic/Biphasic Waveform	Monopolar/Bipolar Electrode Configuration	Reported Outcome
[33]	Porcine	3 months	Not specified	Not specified	Monophasic	Not specified	(1) IRE ablation: PV ostial diameter decreased 11 ± 10% directly after ablation but had increased 19 ± 11% after 3 months. (2) RF ablation: PV ostial diameter decreased 23 ± 15% directly after ablation and remained 7 ± 17% smaller after 3 months than pre-ablation diameter, despite a 21 ± 7% increase in heart size during aging from 6 to 9 months.
[34]	Canine	N/A	Not specified	F- 1 Hz.	Not specified	Bipolar	(1) No evidence of collateral damage to surrounding structures. (2) Ventricular arrhythmias can occur during DC application and are more likely with use of higher energy.
[35]	Canine	27 days	2 kV/cm	PD- 100 µs, PF- 100.	Not specified	Bipolar	(1) No significant PV stenosis or oesophageal injury occurred.
[3]	Porcine	N/A	500 V; 1200 V/cm	PD- 50 µs, F- 10 Hz, PF- 50.	Not specified	Not specified	(1) IREis effective for creating lesions on PV tissue.
[36]	Porcine	35 days	2200 V	PD- <60 s	Biphasic	Bipolar	(1) Fibrous tissue homogeneously replaced myocytes. (2) When present, nerve fascicles and vasculature were preserved within surrounding fibrosis.
[37]	Canine ex vivo	N/A	750–2500 V; 250–833 V/cm	PD- 200 µs, F- 1 Hz, PF- 10	Biphasic	Not specified	(1) Delivery of IRE energy significantly reduced the window of vulnerability to ventricular arrhythmia. (2) No evidence of myocardial damage.

3. Electroporation as an Ablative Approach

Catheter-based electroporation (EPo) using monophasic pulses was first employed with cardiac tissue in the 1980s but it was found to be associated with negative side effects such as the induction of an electrically isolating “vapor globe” resulting in a spark (arcing), followed by an explosion and damaging pressure waves [14][15][38]. Serious complications such as barotrauma and a pro-arrhythmic effect saw voltage-based energy systems superseded by RF ablation [38][39]. However, Ahsan et al. demonstrated that the cautious use of electroporation at lower energies could successfully avoid arcing and produce sufficient therapeutic lesions [40]. Modern voltage-based systems typically employ pulsed electric fields (PEFs) [41][42]. Ablation based on EPo is growing in popularity as an alternative to thermal ablation and causes a biophysical phenomenon to arise following the application of PEF [2][16]. These electric fields induce irreparable pore

formation in cell membranes [3]. As a result, so-called PFA is considered minimally thermal and creates more predictable and controllable lesions, with minimal interaction with blood flow.

Since 2005, both irreversible (IRE) and reversible (RE) EPo has received considerable attention as a method of disrupting cell membranes for drug delivery or inducing selective cell death, respectively [43]. Both IRE and RE have the potential to be tissue-specific in terms of lethal or effective thresholds, with extracellular and endothelial structures commonly remaining intact following exposure to electric fields [44][17]. The permanent opening of nanopores in cell membranes activates intracellular molecular pathways, increases ionic and molecular transport, resulting in an overall disruption of the cell membrane and intracellular homeostasis [43][45][46]. Exposure to sufficiently large field strength results in IRE, and permanent damage and cell death ensues due to localized rearrangement within membrane structures, while supporting structures remain unscathed [9][47][48][18][19]. RE, in contrast, only transiently opens membrane pores, maintaining cell viability, and is commonly employed in the targeted delivery of drugs and nucleotides [43].

The extent and targeting of ablation with IRE can be controlled at least to some degree by changing parameters such as pulse amplitude, frequency, duration of the application and pulse number [2][20]. The lethal thresholds for many cell types have been reported based on these parameters; however, many contradictory data exist as it is still an active area of ongoing research. On the face of it, short exposures and microsecond EPo impulses can be used for biomedical applications aimed at drug delivery and gene transfer, while more prolonged impulses are related to cellular injury and ablation by IRE [47][49][50]. The shape of the applied pulse is an under-explored, and in many cases a poorly documented, parameter that has not received the same degree of experimental testing as amplitude, frequency and others (Table 1 and Table 2). Using a lung cell line, Kotnik et al. demonstrated that of the parameters used to describe pulse shape, the major factor determining electropermeabilization was the amount of time the pulse amplitude exceeded a certain threshold value [51]. They suggest that any differences observed between various pulse shapes may in fact be reflecting the difference in time the pulse is above the critical threshold for that cell type. Meanwhile, Stankevic et al. reported that it is the pulse shape and total energy input that contribute to the efficiency of IRE [52]. Sano et al. (2017) reported that asymmetric waveforms have significantly lower IRE thresholds compared to equivalent symmetrical waveforms, at least for neuroblastoma cells in vitro [53]. Both symmetrical and asymmetrical biphasic pulses have proven effective in IRE cardiac ablation procedures in both animals and a small number of pilot human trials [15][54][55][21][56]. Overall, asymmetric waveforms appear to produce more effective pore opening than symmetric pulses, possibly due to the different amplitudes of their phases. We recommend that all elements of pulse profile need to be reported, according to a set of recommended guidelines, as the extent that pulse shape contributes towards the safety and efficacy for AF treatment with IRE is unclear [57]. Overall, this is an area that requires substantial and more fundamental research before it can become part of standard clinical application [21].

Table 2. Comparison of clinical IRE studies on cardiac tissue.

Ref.	Follow-Up	Energy	Parameters	Monophasic/Biphasic Waveform	Monopolar/Bipolar Electrode Configuration	Reported Outcome Reported Outcome
[56]	N/A	900–2500 V	PF- 3.	Not specified	Bipolar	(1) PEF is a safe method for treating AF both endocardially and epicardially. (2) No incidences of atrial or ventricular arrythmia during procedure. (3) No collateral damage or PV stenosis recorded.
[58]	4 months	900–1000 V	Not specified	Monophasic	Bipolar	(1) Acute PVI achieved in 100% of patients using 6.4 ± 2.3 applications. (2) No injury to oesophagus or phrenic nerve.
[59]	12 months	0.011 ± 0.006 mV	PD- 3–5 s	Biphasic	Bipolar	(1) No adverse effects recorded related to PEF. (2) Freedom from AF was 94.4 ± 3.2%.
[60]	N/A	2154 ± 59 V	Not specified	Monophasic	Monopolar	(1) Acute bidirectional electrical PVI achieved in all 40 PVs. (2) No PV reconnections occurred during waiting period (30 min).

Ref.	Follow-Up	Energy	Parameters	Monophasic/Biphasic Waveform	Monopolar/Bipolar Electrode Configuration	Reported Outcome Reported Outcome
[61]	3 months	900–1000 V	Not specified	Monophasic	Monopolar and Bipolar	(1) No change (0%) in PV diameter and no stenosis in PFA patients, but reduction in diameter in 32.5% of patients who received RFA.

More recently, the field has focused on pulse timing issues [22]. With nanosecond-PEFs in particular, this has been shown to improve the controllability of pore size. Short duration nsPEFs have been shown to minimise the electrophoretic effects associated with cell membrane transport [22][23]. When compared with longer pulse durations, shorter durations are reported to limit solute movement, overall reducing the osmotic imbalance and improving cell targeting with PEF exposure. nsPEF stimuli are too short to induce capacitive charging and instead aim to influence displacement currents over conduction currents [22]. Elementally, every cell behaves independently, deeming intercellular electric connections ineffective on membrane charging [24]. However, the mechanism by which such short stimuli can influence pore opening is still not fully understood and is the subject of ongoing research [22].

3. Conclusions

IRE has seen its stock rise substantially as a therapeutic intervention in recent decades and there has been much interest in its safety and feasibility for use on cardiac tissue. While significant advances have been made based on animal studies, particularly involving porcine and canine models, and preliminary parameters have been developed for use in humans (Table 2), much optimisation remains to be achieved. Further testing and fine-tuning are required to adapt and potentially individualise these parameters for specific patients or patient groups, while ensuring precise delivery of energy to achieve efficient EP ablation. There is significant room for the development of more complex representative in vitro model systems that incorporate both functional and histological outcomes, that are multi-cellular and more easily translatable. This will facilitate rapid development of pulse parameters and potentially catheter design by looking at the catheter not just to deliver energy, but to also provide feedback on target site and success of the ablation.

Similarly, while there are substantial preclinical data for IRE from animal models, the number of clinical trials is limited. Studies completed to date include small cohorts of approximately eighty patients with varying follow-up times of 3, 4 and 12 months [58][59][61]. Therefore, not only larger, multicentre trials are required to analyse the effects of IRE but also long-term evaluation of the permanence of the ablation.

Lesions are difficult to investigate in human studies, thus, most information is to be acquired regarding the true depth and volume of lesions is collected from animal studies. Follow-up times of preclinical trials generally exceed no longer than 3 or 4 months (Table 1). Similarly, long-term studies would challenge the durability of lesions in humans and examine any relapse to the electrical or structural induced CVD originally treated by IRE. Another limitation to current IRE trials is the lack of consistency between experiments. Some studies are limited to one energy magnitude, while others either use smaller or greater magnitudes on different sized animals (Table 1). While there are few published clinical trials related to the use of IRE on cardiac tissue, preclinical studies provide a promising baseline representation of its use. IRE bypasses many of the complications and drawbacks of the more commonly used thermal ablation modalities. With further improvements and refinement of parameter specifics, IRE may prove to be the gold standard for ablative CVD therapy.

References

1. Heart Disease Facts|cdc.gov. Available online: (accessed on 9 June 2020).
2. Zager, Y.; Kain, D.; Landa, N.; Leor, J.; Maor, E. Optimization of irreversible electroporation protocols for in-vivo myocardial decellularization. PLoS ONE 2016, 11.
3. Jiang, C.; Goff, R.; Patana-anake, P.; Iaizzo, P.A.; Bischof, J. Irreversible electroporation of cardiovascular cells and tissues. J. Med. Devices Trans. ASME 2013, 7.
4. Avazzadeh, S.; McBride, S.; O'Brien, B.; Coffey, K.; Elahi, A.; O'Halloran, M.; Soo, A.; Quinlan, L.R. Ganglionated Plexi Ablation for the Treatment of Atrial Fibrillation. J. Clin. Med. 2020, 9, 3081.
5. Holmes, D.R.; Valeti, U.S.; Nishimura, R.A. Alcohol septal ablation for hypertrophic cardiomyopathy: Indications and technique. Catheter. Cardiovasc. Interv. 2005, 66, 375–389.

6. Nagueh, S.F.; Groves, B.M.; Schwartz, L.; Smith, K.M.; Wang, A.; Bach, R.G.; Nielsen, C.; Leya, F.; Buergler, J.M.; Rowe, S.K.; et al. Alcohol septal ablation for the treatment of hypertrophic obstructive cardiomyopathy: A multicenter north american registry. J. Am. Coll. Cardiol. 2011, 58, 2322–2328.
7. Wei, C.; Qian, P.; Tedrow, U.; Mak, R.; Zei, P.C. Non-invasive stereotactic radioablation: A new option for the treatment of ventricular arrhythmias. Arrhythmia Electrophysiol. Rev. 2019, 8, 285–293.
8. Chiu, M.H.; Mitchell, L.B.; Ploquin, N.; Faruqi, S.; Kuriachan, V.P.; Chiu, M. Review of Stereotactic Arrhythmia Radioablation Therapy for Cardiac Tachydysrhythmias. CJC Open 2020, 3, 236–247.
9. Lavee, J.; Onik, G.; Rubinsky, B. A Novel Nonthermal Energy Source for Surgical Epicardial Atrial Ablation: Irreversible Electroporation Single cell manipulation and electroporation View project Isochoric Freezing: A new frontier for cryopreservation View project. Heart Surg. Forum 2007, 10, 96–101.
10. Njeim, M.; Bogun, F. Selecting the appropriate ablation strategy: The role of endocardial and/or epicardial access. Arrhythmia Electrophysiol. Rev. 2015, 4, 184–188.
11. Krassowska, W. Effects of Electroporation on Transmembrane Potential Induced by Defibrillation Shocks. Pacing Clin. Electrophysiol. 1995, 18, 1644–1660.
12. Hirano, M.; Yamamoto, H.; Hasebe, Y.; Fukuda, K.; Morosawa, S.; Amamizu, H.; Ohyama, K.; Uzuka, H.; Takayama, K.; Shimokawa, H. Development of a Novel Shock Wave Catheter Ablation system—A Validation Study in Pigs in Vivo. EP Eur. 2018, 20, 1856–1865.
13. Du Pre, B.C.; van Driel, V.J.; van Wessel, H.; Loh, P.; Doevendans, P.A.; Goldschmeding, R.; Wittkampf, F.H.; Vink, A. Minimal Coronary Artery Damage by Myocardial Electroporation Ablation. EP Eur. 2013, 15, 144–149.
14. Neven, K.; van Driel, V.; van Wessel, H.; van Es, R.; du Pré, B.; Doevendans, P.A.; Wittkampf, F. Safety and feasibility of closed chest epicardial catheter ablation using electroporation. Circ. Arrhythm. Electrophysiol. 2014, 7, 913–919.
15. Stewart, M.T.; Haines, D.E.; Verma, A.; Kirchhof, N.; Barka, N.; Grassl, E.; Howard, B. Intracardiac pulsed field ablation: Proof of feasibility in a chronic porcine model. Heart Rhythm 2019, 16, 754–764.
16. Sugrue, A.; Vaidya, V.R.; Livia, C.; Padmanabhan, D.; Abudan, A.; Isath, A.; Witt, T.; DeSimone, C.V.; Stalboerger, P.; Kapa, S.; et al. Feasibility of selective cardiac ventricular electroporation. PLoS ONE 2020, 15.
17. Wittkampf, F.H.; Van Driel, V.J.; Van Wessel, H.; Vink, A.; Hof, I.E.; GrÜndeman, P.F.; Hauer, R.N.; Loh, P. Feasibility of electroporation for the creation of pulmonary vein ostial lesions. J. Cardiovasc. Electrophysiol. 2011, 22, 302–309.
18. Semenov, I.; Zemlin, C.; Pakhomova, O.N.; Xiao, S.; Pakhomov, A.G. Diffuse, non-polar electropermeabilization and reduced propidium uptake distinguish the effect of nanosecond electric pulses. Biochim. Biophys. Acta Biomembr. 2015, 1848, 2118–2125.
19. Al-Khadra, A.; Nikolski, V.; Efimov, I.R. The role of electroporation in defibrillation. Circ. Res. 2000, 87, 797–804.
20. Hong, J.; Stewart, M.T.; Cheek, D.S.; Francischelli, D.E.; Kirchhof, N. Cardiac ablation via electroporation. In Proceedings of the 31st Annual International Conference of the IEEE Engineering in Medicine and Biology Society: Engineering the Future of Biomedicine, EMBC 2009, Minneapollis, MN, USA, 3–6 September 2009; Volume 2009, pp. 3381–3384.
21. Van Es, R.; Konings, M.K.; Du Pré, B.C.; Neven, K.; Van Wessel, H.; Van Driel, V.J.H.M.; Westra, A.H.; Doevendans, P.A.F.; Wittkampf, F.H.M. High-frequency irreversible electroporation for cardiac ablation using an asymmetrical waveform. Biomed. Eng. Online 2019, 18.
22. Semenov, I.; Grigoryev, S.; Neuber, J.U.; Zemlin, C.W.; Pakhomova, O.N.; Casciola, M.; Pakhomov, A.G. Excitation and injury of adult ventricular cardiomyocytes by nano- to millisecond electric shocks. Sci. Rep. 2018, 8, 1–12.
23. Azarov, J.E.; Semenov, I.; Casciola, M.; Pakhomov, A.G. Excitation of murine cardiac myocytes by nanosecond pulsed electric field. J. Cardiovasc. Electrophysiol. 2019, 30, 392–401.
24. Xie, F.; Varghese, F.; Pakhomov, A.G.; Semenov, I.; Xiao, S.; Philpott, J.; Zemlin, C. Ablation of Myocardial Tissue With Nanosecond Pulsed Electric Fields. PLoS ONE 2015, 10, e0144833.
25. Padmanabhan, D.; Naksuk, N.; Killu, A.K.; Kapa, S.; Witt, C.; Sugrue, A.; Desimon, C.V.; Madhavan, M.; de Groot, J.R.; O'Brien, B.; et al. Electroporation of epicardial autonomic ganglia: Safety and efficacy in medium-term canine models. J. Cardiovasc. Electrophysiol. 2019, 30, 607–615.
26. Koruth, J.; Kuroki, K.; Iwasawa, J.; Enomoto, Y.; Viswanathan, R.; Brose, R.; Buck, E.D.; Speltz, M.; Dukkipati, S.R.; Reddy, V.Y. Preclinical Evaluation of Pulsed Field Ablation: Electrophysiological and Histological Assessment of Thoracic Vein Isolation. Circ. Arrhythmia Electrophysiol. 2019, 12.
27. Kim, S.C.; Vasanji, A.; Efimov, I.R.; Cheng, Y. Spatial distribution and extent of electroporation by strong internal shock in intact structurally normal and chronically infarcted rabbit hearts. J. Cardiovasc. Electrophysiol. 2008, 19, 1080–1089.

28. Xie, F.; Zemlin, C.W. Effect of Twisted Fiber Anisotropy in Cardiac Tissue on Ablation with Pulsed Electric Fields. PLoS ONE 2016, 11, e0152262.

29. Neven, K.; Van Driel, V.; Van Wessel, H.; Van Es, R.; Doevendans, P.A.; Wittkampf, F. Myocardial Lesion Size after Epicardial Electroporation Catheter Ablation After Subxiphoid Puncture. Circ. Arrhythmia Electrophysiol. 2014, 7, 728–733.

30. Neven, K.; Van Driel, V.; Van Wessel, H.; Van Es, R.; Doevendans, P.A.; Wittkampf, F. Epicardial linear electroporation ablation and lesion size. Hear. Rhythm 2014, 11, 1465–1470.

31. Wittkampf, F.H.M.; Van Driel, V.J.; Van Wessel, H.; Neven, K.G.E.J.; Gründeman, P.F.; Vink, A.; Loh, P.; Doevendans, P.A. Myocardial lesion depth with circular electroporation ablation. Circ. Arrhythmia Electrophysiol. 2012, 5, 581–586.

32. Desimone, C.V.; Ebrille, E.; Syed, F.F.; Mikell, S.B.; Suddendorf, S.H.; Wahnschaffe, D.; Ladewig, D.J.; Gilles, E.J.; Danielsen, A.J.; Holmes, D.R.; et al. 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. Transl. Res. 2014, 164, 508–514.

33. Van Driel, V.J.H.M.; Neven, K.G.E.J.; Van Wessel, H.; Du Pré, B.C.; Vink, A.; Doevendans, P.A.F.M.; Wittkampf, F.H.M. Pulmonary vein stenosis after catheter ablation electroporation versus radiofrequency. Circ. Arrhythmia Electrophysiol. 2014, 7, 734–738.

34. Madhavan, M.; Venkatachalam, K.L.; Swale, M.J.; Desimone, C.V.; Gard, J.J.; Johnson, S.B.; Suddendorf, S.H.; Mikell, S.B.; Ladewig, D.J.; Nosbush, T.G.; et al. Novel Percutaneous Epicardial Autonomic Modulation in the Canine for Atrial Fibrillation: Results of an Efficacy and Safety Study. PACE - Pacing Clin. Electrophysiol. 2016, 39, 407–417.

35. Witt, C.M.; Sugrue, A.; Padmanabhan, D.; Vaidya, V.; Gruba, S.; Rohl, J.; DeSimone, C.V.; Killu, A.M.; Naksuk, N.; Pederson, J.; et al. Intrapulmonary vein ablation without stenosis: A novel balloon-based direct current electroporation approach. J. Am. Heart Assoc. 2018, 7.

36. Koruth, J.S.; Kuroki, K.; Iwasaw1, J.; Viswanathan, R.; Richard; Brose; Buck, E.D.; Donskoy, E.; Dukkipati, S.R.; Reddy, V.Y. Endocardial Ventricular Pulsed Field Ablation: A Proof-Of-Concept Preclinical Evaluation. EP Eur. 2020, 22, 434–439.

37. Livia, C.; Sugrue, A.; Witt, T.; Polkinghorne, M.D.; Maor, E.; Kapa, S.; Lehmann, H.I.; DeSimone, C.V.; Behfar, A.; Asirvatham, S.J.; et al. Elimination of Purkinje Fibers by Electroporation Reduces Ventricular Fibrillation Vulnerability. J. Am. Heart Assoc. 2018, 7, e009070.

38. Coltorti, F.; Bardy, G.H.; Reichenbach, D.; Greene, H.L.; Thomas, R.; Breazeale, D.G.; Alferness, C.; Ivey, T.D. Catheter-mediated electrical ablation of the posterior septum via the coronary sinus: Electrophysiologic and histologic observations in dogs. Circulation 1985, 72, 612–622.

39. Nakagawa, H.; Jackman, M. Electroporation (revival of direct current ablation) new approach for increasing epicardial ablation safety in close proximity to a coronary artery. Circ. Arrhythmia Electrophysiol. 2014, 7, 779–780.

40. Ahsan, A.J.; Cunningham, D.; Rowland, E.; Rickards, A.F. Catheter Ablation without Fulguration: Design and Performance of a New System. Pacing Clin. Electrophysiol. 1989, 12, 1557–1561.

41. Wittkampf, F.H.M.; van Es, R.; Neven, K. Electroporation and its Relevance for Cardiac Catheter Ablation. JACC Clin. Electrophysiol. 2018, 4, 977–986.

42. Tung, L.; Tovar, O.; Neunlist, M.; Jain, S.K.; O'neill, R.J. Effects of Strong Electrical Shock on Cardiac Muscle Tissue. Ann. N. Y. Acad. Sci. 1994, 720, 160–175.

43. Davalos, R.V.; Mir, L.M.; Rubinsky, B. Tissue ablation with irreversible electroporation. Ann. Biomed. Eng. 2005, 33, 223–231.

44. Maor, E.; Ivorra, A.; Mitchell, J.J.; Rubinsky, B. Vascular smooth muscle cells ablation with endovascular nonthermal irreversible electroporation. J. Vasc. Interv. Radiol. 2010, 21, 1708–1715.

45. Wojtaszczyk, A.; Caluori, G.; Pešl, M.; Melajova, K.; Stárek, Z. Irreversible electroporation ablation for atrial fibrillation. J. Cardiovasc. Electrophysiol. 2018, 29, 643–651.

46. Frandsen, S.K.; Gissel, H.; Hojman, P.; Tramm, T.; Eriksen, J.; Gehl, J. Direct therapeutic applications of calcium electroporation to effectively induce tumor necrosis. Cancer Res. 2012, 72, 1336–1341.

47. Weaver, J.C. Electroporation of cells and tissues. IEEE Trans. Plasma Sci. 2000, 28, 24–33.

48. Neumann, E.; Rosenheck, K. Permeability changes induced by electric impulses in vesicular membranes. J. Membr. Biol. 1972, 10, 279–290.

49. Vižintin, A.; Vidmar, J.; Ščančar, J.; Miklavčič, D. Effect of interphase and interpulse delay in high-frequency irreversible electroporation pulses on cell survival, membrane permeabilization and electrode material release. Bioelectrochemistry

2020, 134, 107523.

50. Andrei, G.; Pakhomov, D.; Miklavcic, M.S.M. Advanced Electroporation Techniques in Biology and Medicine. Available online: (accessed on 27 March 2020).
51. Kotnik, T.; Pucihar, G.; Reberšek, M.; Miklavčič, D.; Mir, L.M. Role of pulse shape in cell membrane electropermeabilization. Biochim. Biophys. Acta Biomembr. 2003, 1614, 193–200.
52. Stankevic, V.; Simonis, P.; Zurauskiene, N.; Stirke, A.; Dervinis, A.; Bleizgys, V.; Kersulis, S.; Balevicius, S. Compact square-wave pulse electroporator with controlled electroporation efficiency and cell viability. Symmetry 2020, 12, 412.
53. Sano, M.B.; Fan, R.E.; Xing, L. Asymmetric Waveforms Decrease Lethal Thresholds in High Frequency Irreversible Electroporation Therapies. Sci. Rep. 2017, 7, 1–13.
54. Polajžer, T.; Dermol-Cerne, J.; Erne, J.; Reberšek, M.; O'connor, R.; Miklavčič, D. Cancellation effect is present in high-frequency reversible and irreversible electroporation. Bioelectrochemistry 2019.
55. Caluori, G.; Odehnalova, E.; Jadczyk, T.; Pesl, M.; Pavlova, I.; Valikova, L.; Holzinger, S.; Novotna, V.; Rotrekl, V.; Hampl, A.; et al. AC Pulsed Field Ablation Is Feasible and Safe in Atrial and Ventricular Settings: A Proof-of-Concept Chronic Animal Study. Front. Bioeng. Biotechnol. 2020, 8, 1374.
56. Reddy, V.Y.; Koruth, J.; Jais, P.; Petru, J.; Timko, F.; Skalsky, I.; Hebeler, R.; Labrousse, L.; Barandon, L.; Kralovec, S.; et al. Ablation of Atrial Fibrillation With Pulsed Electric Fields: An Ultra-Rapid, Tissue-Selective Modality for Cardiac Ablation. JACC Clin. Electrophysiol. 2018, 4, 987–995.
57. Cemazar, M.; Sersa, G.; Frey, W.; Miklavcic, D.; Teissié, J. Recommendations and requirements for reporting on applications of electric pulse delivery for electroporation of biological samples. Bioelectrochemistry 2018, 122, 69–76.
58. Reddy, V.Y.; Neuzil, P.; Koruth, J.S.; Petru, J.; Funosako, M.; Cochet, H.; Sediva, L.; Chovanec, M.; Dukkipati, S.R.; Jais, P. Pulsed Field Ablation for Pulmonary Vein Isolation in Atrial Fibrillation. J. Am. Coll. Cardiol. 2019.
59. Reddy, V.Y.; Anter, E.; Rackauskas, G.; Peichl, P.; Koruth, J.S.; Petru, J.; Funasako, M.; Minami, K.; Natale, A.; Jaïs, P.; et al. A Lattice-Tip Focal Ablation Catheter that Toggles Between Radiofrequency and Pulsed Field Energy to Treat Atrial Fibrillation: A First-in-Human Trial. Circ. Arrhythmia Electrophysiol. 2020, 13.
60. Loh, P.; Van Es, R.; Groen, M.H.A.; Neven, K.; Kassenberg, W.; Wittkampf, F.H.M.; Doevendans, P.A. Pulmonary vein isolation with single pulse irreversible electroporation: A first in human study in 10 patients with atrial fibrillation. Circ. Arrhythmia Electrophysiol. 2020, 13, 1083–1091.
61. Kuroki, K.; Whang, W.; Eggert, C.; Lam, J.; Leavitt, J.; Kawamura, I.; Reddy, A.; Morrow, B.; Schneider, C.; Petru, J.; et al. Ostial Dimensional Changes After Pulmonary Vein Isolation: Pulsed Field Ablation vs Radiofrequency Ablation. Hear. Rhythm 2020.