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MULTIMEDIA REPORT

Irreversible electroporation for catheter-based cardiac ablation: a systematic review of the preclinical experience

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Abstract

Introduction Irreversible electroporation (IRE) utilizing high voltage pulses is an emerging strategy for catheter-based cardiac ablation with considerable growth in the preclinical arena.

Methods A systematic search for articles was performed from three sources (PubMed, EMBASE, and Google Scholar). The primary outcome was the efficacy of tissue ablation with characteristics of lesion formation evaluated by histologic analysis. The secondary outcome was focused on safety and damage to collateral structures.

Results Sixteen studies met inclusion criteria. IRE was most commonly applied to the ventricular myocardium ($n = 7/16$, 44%) by a LifePak 9 Defibrillator ($n = 9/16$, 56%), NanoKnife Generator ($n = 2/16$, 13%), or other custom generators ($n = 5/16$, 31%). There was significant heterogeneity regarding electroporation protocols. On histological analysis, IRE was successful in creating ablation lesions with variable transmurality depending on the electric pulse parameters and catheter used.

Conclusion Preclinical studies suggest that cardiac tissue ablation using IRE shows promise in delivering efficacious, safe lesions.

Keywords Cardiac ablation · Irreversible electroporation · Pulsed electric field · Atrial fibrillation · Arrhythmias · Catheter ablation · Translational studies

Abbreviations

CA	Coronary arteries
DC	Direct current
ECG	Electrocardiogram
IRE	Irreversible electroporation
PV	Pulmonary vein
RF	Radiofrequency
SVC	Superior vena cava
VF	Ventricular fibrillation

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1 Introduction

Since it was first performed in 1969, cardiac ablation has experienced numerous innovations and has evolved immensely [1]. Historically, ablation was performed for the treatment of supraventricular tachycardia in patients with accessory pathways and pre-excitation syndromes, with its success in patients with refractory arrhythmias sparking vast growth and expanded indications. Today, cardiac ablation is regularly used for the treatment of atrial flutter [2], atrial fibrillation [3–5], and ventricular arrhythmias [6, 7].

The goal of ablation is to destroy the underlying arrhythmogenic tissue and create permanent lesions that are both transmural and contiguous. Energy sources used to create these lesions historically have evolved. Direct current (DC) was the initial energy source used [8–10]; however, inconsistency with lesion formation, barotrauma from arcing and

recurrence of arrhythmias, drove physicians and engineers to both develop and investigate alternative energy modalities. This ultimately paved the way for radiofrequency (RF) energy, currently the most commonly used energy source [11, 12]. RF creates lesions by resistive heating of tissue and subsequent heat conduction to deeper tissue. While reasonably efficacious, it can be associated with undesirable effects to vital structures, stemming from its thermal nature of action, not only on the applied tissue but also to vital collateral structures. In particular, thermal heat during ablation with RF is responsible for injury to the esophagus (which predisposes for atrioesophageal fistula formation) [13, 14], phrenic nerve damage [15], and formation of coagulum/thrombus with subsequent risk for thromboembolism and both overt [16] and silent cerebral infarcts/lesions [17, 18]. Cryothermal ablation is another widely employed ablation modality that is contrastingly different to RF. It ablates tissue by removing heat which results in tissue cooling and ice formation [19]. However, cryothermal ablation, like RF, is also associated with complications including esophageal fistula [20], pulmonary vein (PV) stenosis [21], phrenic nerve palsy [22], and potential lung hemoptysis [23]. Although both these energy sources for ablation are largely efficacious, there has been a desire to try alternative ablation energies to improve ablation safety.

The emergence, or somewhat resurgence, of DC has seen growth in its application in the preclinical arena as a means for creating ablation lesions via irreversible electroporation (IRE) of tissue. The use of DC in a pulsed form creates a local electric field which affects the lipid bilayer permeability of the cellular membrane inducing the formation of nano-scale defects or pores which leads to the permeabilization of cells. Depending upon the electric pulse delivery settings (e.g., pulse duration, voltage, frequency), this can be reversible, meaning the cell can survive because of the re-establishment of cell membrane integrity and electrical homeostasis, or irreversible leading to cell death [24]. IRE is a growing, well-established FDA approved treatment modality for solid tumors [25–28] and was recently approved for the treatment of pancreatic cancer [29]. It is an alluring method for cardiac ablation, particularly when compared to RF, as it may create ablation lesions without the consequences of thermal damage and enable preservation of surrounding collateral structures [30, 31]. With the potential advantages of IRE over current ablation modalities, there has been considerable growth in preclinical animal publications and very recently, there was publication of the first in human acute data [32]. Considering this growth and recent translation to humans, we sought to conduct a systematic review of current preclinical animal studies employing cardiac IRE. This review aims to synthesize and provide an update on the efficacy and safety of cardiac IRE with the ultimate goal of helping optimize future preclinical experiments and ablation approaches. Also, it will help identify current knowledge gaps which could serve as a vehicle to usher increased translation from preclinical animal studies to human clinical trials.

2 Methods

The review methodology was pre-specified and documented using SYRCLE's (Systematic Review Centre for Laboratory Animal Experimentation) systematic review protocol for animal intervention studies [33] and was performed in line with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyze) statement [34].

2.1 Search strategy

Preclinical studies on the use of cardiac IRE as an ablation modality were identified by comprehensive searches using three sources (PubMed, EMBASE, and Google Scholar); we used the search components "cardiac," "irreversible electroporation," "ablation," and "animal" (for full search strategy see Supplemental Table 1). The literature was reviewed up to March 1, 2018. No limits were applied to language. Additional citations were assembled from the reference lists of related papers and review articles.

2.2 Study selection

After removal of duplicates studies, two investigators (A.S. and V.V.) independently screened all titles and abstracts to identify studies meeting the inclusion criteria. Studies were included if it was an animal model (*in vivo* or *ex vivo*) and if the study met ≥ 1 of the following criteria: (1) assessed the effect of IRE on cardiac tissue (either myocardium, nerves, ganglia); (2) evaluated the effect of IRE on collateral cardiac structures (phrenic nerve, esophagus, vagus nerve); (3) reported safety outcomes on cardiac IRE application. Meeting abstracts were not included in this review. Full text of all potentially eligible studies was retrieved and independently assessed for eligibility by two investigators (A.S and V.V) with disagreements resolved by consensus.

2.3 Outcomes assessed

The primary outcome assessed was lesion formation (size and transmurality) on histology. Secondary outcome included ablation safety through/by evaluating/assessing/observing damage to collateral structures.

2.4 Data abstraction

Study characteristics were extracted by one reviewer (A.S.) and checked for inconsistencies by a second reviewer (V.V.), with disagreements resolved by consensus. For each study, we

extracted data on a standardized extraction form which included the animal model used, type of tissue targeted, source of high voltage pulses, type of ablation device used (including electrode size, spacing, shape), high voltage delivery (or pulsed electric field) parameters (pulse duration, pulse frequency, number of pulses, voltage applied), as well as bibliographic details (1st author, year, title, journal). If data were presented graphically only, we extracted data using a digital screen ruler, capable of measurement to 0.1 mm.

2.5 Quality assessment

We employed the ARRIVE checklist [35] to assess methodological quality and bias. The ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines are intended to improve the reporting of research using animals and consists of a checklist of 20 items describing information that all scientific publications should include. Each study was given a quality score out of a possible total of 20 points, and the group median was calculated. Two independent investigators (A.S. and V.V) performed a quality assessment of all included studies and resolved disagreements by consensus.

2.6 Statistical analysis

Owing to considerable heterogeneity in the reported methods and data, a meta-analysis was not feasible. As a result, no statistical examination was performed or formal testing of bias across multiple studies.

3 Results

3.1 Study characteristics

From 68 potentially eligible studies, 23 were retrieved for full-text evaluation after screening citations by title and abstract, and subsequently, 16 studies met the inclusion criteria and were included in this review (Fig. 1) [36–51]. All studies were interventional cohort studies, and 6 (38%) studies specified a control group. In total, 171 animals were studied with swine as the most common preclinical model ($n = 10/16$, 63%). Five were acute studies, 10 were chronic survival studies, and one study involved both acute and chronic models. High voltage pulses for IRE was applied most commonly to the ventricular myocardium ($n = 7/16$, 44%) followed by atrial tissue/pulmonary veins ($n = 6/16$, 38%), coronary arteries ($n = 1/16$, 6%), esophagus ($n = 1/16$, 6%), phrenic nerve ($n = 1/16$, 6%), and cardiac ganglia ($n = 1/16$, 6%). All preclinical studies involved healthy animal models. Further characteristics of these 16 studies are highlighted in Table 1.

3.2 Quality of studies

According to the ARRIVE guidelines, the median score for quality of studies was 18 (range 14–20). At times although it was not explicitly stated what the primary and secondary outcomes of the study were, this could be generally inferred.

3.3 Electroporation: delivery and protocols

A total of 320 ablations were performed across the 16 studies (Table 2), with further more detailed information provided in Supplemental Table 2. High voltage electric pulses were delivered by a LifePak 9 Defibrillator in 9 studies ($n = 9/16$, 56%), the NanoKnife generator in two studies ($n = 2/16$, 13%), and other generators in five studies ($n = 5/16.31\%$). Fourteen different catheter types were used for ablation across 16 studies, with six studies testing two or more different catheters. The most common catheter type was a circular multi-electrode ring catheter ($n = 7/16$, 44%), followed by a linear ablation catheter ($n = 4/16$, 25%), and other custom prototype catheters (5/16, 31%). As there are no commercially developed catheters for the specific delivery of electric pulses, most studies employed currently used catheters for radiofrequency ablation delivery which were modified as needed or developed new prototype catheters (e.g., balloon or linear catheters).

There was significant heterogeneity regarding both electroporation protocols and reporting of protocols across all studies. Pulse duration and number of pulses was consistently reported and varied from 20 μs to 6 msec and 1–200, respectively. Pulse repetition frequency was rarely reported, with 13 studies (81%) not reporting this. In the three studies that it was published, the frequency ranged between 1 and 5 Hz. The amount of energy delivered was heterogeneous across all studies with many different units reported, with 9 (56%) studies reporting Joules, 4 (25%) voltage, 2 (13%) electric current, and 1 (6%) study did not indicate the specific parameters of electric pulses applied. Two studies reported the voltage-to-distance ratio (V/cm).

3.4 Lesion histology (Table 3)

3.4.1 Ventricular myocardium

A total of six studies applied high voltages pulses to the ventricular epicardium and one study to the endocardium. When applied to ventricular epicardium in acute studies, changes were not observed macroscopically. Chronic survival studies showed that the delivery of energy to the epicardium often resulted in the formation of a white lesion that was sharply demarcated from the surrounding tissue. There was some purple discoloration (bruising) when a linear suction device was

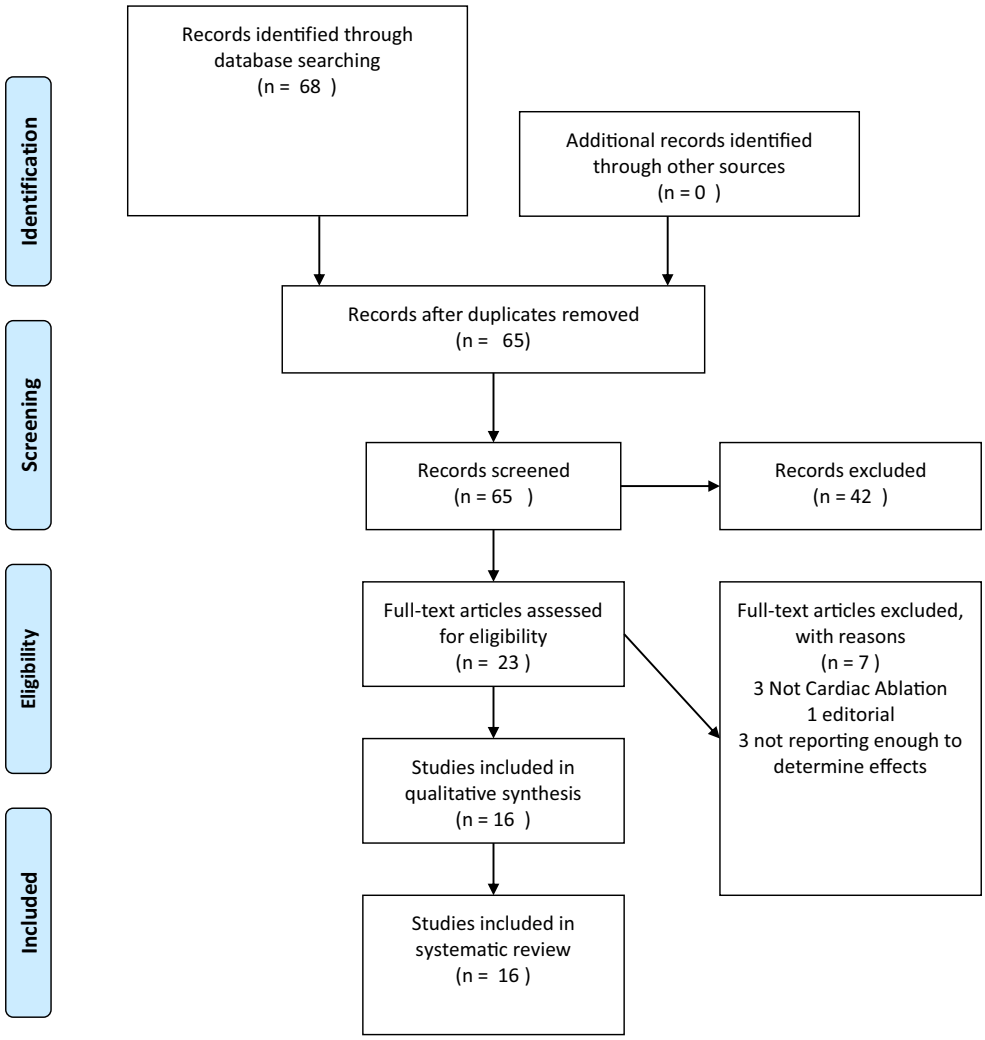

Fig. 1 PRISMA statement

used. Histologically, destruction of cardiac myocytes and connective tissue with loose collagen fibers remained. The higher the energy, i.e., higher amplitude of pulses or longer pulses or greater number of pulses applied, the larger the lesion and the more likely it was transmural, but variations in protocol delivery and inconsistency in reported "energy units" prohibit comparison analysis.

3.4.2 Atrial tissue

Six studies have applied high voltage pulses to atrial tissue. When IRE was applied in the superior vena cava (SVC), 2 out of 3 studies (66%) observed a grossly visible lesion. When applied to the pulmonary vein tissue, this was not the case, and no acute gross macroscopic changes were identified. On histology, decellularization was observed with only collagen scaffolding remaining. Similar to the ventricular epicardium, the higher the energy (higher amplitude, greater number of pulses), the greater the lesion size and transmurality, but again variations in protocol delivery and reported energy units prohibit comparison analysis.

3.4.3 Coronary arteries

In the two studies that reported on high voltage pulses to the coronary arteries (CA) this resulted in varying degrees of intimal hyperplasia. Although mild narrowing was noted, there was no significant stenosis observed, and the vessel was generally unaffected.

3.4.4 Esophagus

Direct application of high voltage pulses to the esophagus was performed in two studies. The first study noted that the lesions were restricted to the muscle layer; the luminal epithelial layer and the lamina muscularis mucosae had no pathological changes. A more recent study by Neven showed that direct esophageal IRE resulted in self-limiting vesicles on the non-keratinizing squamous epithelium at the ablation site. After 7-

Table 1 Characteristics of 16 studies

Study	Author	Year	Tissue type	Animal	Weight (kg, unless stated)	Animal number	Ablation lesions	Control/ sham group	Follow-up (weeks)	Primary outcome	Secondary outcome
1	Lavee [39]	2007	Atrial tissue	Swine	–	5	10	No	Acute model (24 h)	Utility of electroporation to create epicardial atrial lesions	
2	Hong [38]	2009	Ventricular epicardium/atrial tissue	Ovine	–	4	33	No	Acute model (24 h)	Develop systems and methods for cardiac electroporation	Evaluate feasibility of its application to cardiac tissues
3	Wittkampf [47]	2011	Atrial tissue	Swine	60–75	15	54	Yes	2 (pilot)- 3 (feasibility)	Feasibility and safety of cardiac electroporation	
4	Wittkampf [46]	2012	Ventricular epicardium	Swine	60–75	5	5	No	3	Investigate magnitude of circular epicardial electroporation application and lesion size	
5	Du Pre [37]	2012	Coronary arteries	Swine	60–75	9	16	Yes	3	Analyze the effect of IRE on coronary arteries	
6	Neven [41]	2014	Ventricular epicardium	Swine	60–75	5	14	No	12	Investigate magnitude of linear epicardial electroporation application and lesion size	
7	Neven [42]	2014	Ventricular epicardium	Swine	60–75	6	15	No	12	Investigate magnitude of epicardial electroporation application and lesion size	
8	van Driel [45]	2014	Pulmonary vein	Swine	60–75	10	10	Yes	12	Investigate response of pulmonary vein to electroporation vs radiofrequency	
9	Neven [43]	2014	Ventricular epicardium	Swine	60–75	6	13	No	12	Investigate safety and feasibility of electroporation in the epicardial space	
10	DeSimone [36]	2014	Pulmonary vein	Canine	30–40	4		Yes	Acute model (24 h)	Evaluate electroporation of pulmonary vein tissue	
11	Van Driel [44]	2015	Phrenic nerve	Swine	60–75	20	19	No	3–13 (4 animals were acute)	Assess for phrenic nerve damage (histological or functional)	
12	Zager [48]	2016	Ventricular epicardium	Rat	270 ± 21 (grams)	45	45	Yes	4	Evaluate safety of cardiac electroporation in a rodent model	Evaluate and compare the potency and graded effect of different electroporation protocols.
13	Madhavan [40]	2016	Cardiac ganglia plexus	Canine	30–40	16	52	Yes	Acute model (24 h)	Demonstrate feasibility of techniques for percutaneous epicardial ablation of cardiac ganglia	
14	Neven [49]	2017	Esophagus	Swine	60–75	8	16	No	8 (3 were 2 days)	Assess for Esophageal Damage	
15	Livia [50]	2018	Purkinje fibers (ventricular myocardium)	Canine	25–40	8	8	No	Acute *ex vivo* model	Assess for Purkinje/fascicular fibers elimination via a non-thermal IRE approach	
16	Witt [51]	2018	Pulmonary vein	Canine	30–40	5	10	No	7–44 (days)	Evaluate the feasibility of IRE for ablating within the PVs without creating PV stenosis or damage to neighboring structures	

and 60-day follow-up, the epithelium normalized entirely. There were no signs of ulceration or other adverse reactions at both day 7 and day 60.

3.4.5 Ganglia

IRE of cardiac ganglia has been shown in one study to be relatively efficacious. In this study, Madhavan was able to successfully target and ablate ganglia in five out of six dogs (83%).

3.4.6 Safety/adverse events

Only one study observed a significant complication related directly to the delivery of cardiac IRE (Table 4). In this event, inadvertent movement of the catheter over the ventricle during electric pulse delivery resulted in ventricular fibrillation (VF) and early demise (delivery of energy was not performed with synchronization). Ten studies (62%) reported no adverse events with either delivery of IRE or the procedure performed. In the other five studies, there were adverse events reported which were related to the procedures itself rather than IRE delivery. There was no suggestion or reported collateral damage to surrounding cardiac structures.

4 Discussion

As IRE gathers considerable interest as an alternative means to perform cardiac ablation, this systematic review of published preclinical data provides critical synthesis and insight into its efficacy and safety. This review is vital in highlighting knowledge gaps, enabling guidance for future preclinical studies and ultimately helps in the progression from preclinical to clinical studies and practice.

4.1 Effectiveness

Overall, IRE can be successfully applied to cardiac tissue and achieve the goal of creating an ablation lesion. Many of the ablation lesions were transmural; however, definitive recommendations on the optimal IRE parameters for creating a transmural ablation lesion are not possible based on current published studies given the significant heterogeneity in reporting and parameters applied. Further, typical defibrillators do not control the applied voltage, but the total applied energy, which is likely to impact repeatability and reproducibility of studies. Future studies should be meticulous in their reporting of these parameters (Table 5). The most eloquent study that provides insight was performed by Zager et al., where different protocols were applied to rat myocardium (final study included 45 rats) which enabled a direct comparison of effects of different parameter changes. While this study is beneficial, we acknowledge that performing this type of experiment on larger animals (canine or swine) would be prohibitively expensive. That said, although common knowledge amongst the electroporation community, this study shows that use of high voltage, longer pulse duration, lower pulse frequency, and a greater number of pulses results in increased tissue damage (and vice versa). However, it ought to be noted that smaller animals may not be as readily translated to parameters suited for larger animals, and importantly humans. While overall we are unable to provide a meta-analysis on efficacy, it is clear that although different studies employ different devices and generators, IRE can create ablation lesions and this alone provides important support and rationale for continued research and study of this ablation modality.

4.2 Safety

The delivery of IRE has been shown to cause both lethal and non-lethal cardiac arrhythmias [52–54]. In our systematic review, we present a large amount of preclinical animal data that suggests that direct cardiac IRE delivery is reasonably safe, with only one lethal arrhythmic event reported across all 16 studies. In this event, inadvertent movement of the catheter over the ventricle during voltage pulse delivery resulted in VF and early demise. Importantly, no electrocardiogram (ECG) synchronization to high voltage electric pulse delivery was performed. ECG synchronization during pulse delivery is a critical tool to mitigate lethal arrhythmic risk in ensuring that the energy is delivered during the absolute refractory period of the cardiac cycle. For example, the delivery of electrical pulses can be synchronized with the electrocardiogram via AccuSync 42, an external R wave triggering device (AccuSync, USA). The AccuSync 42 detects the R wave of each individual heartbeat early on the ascending slope of the R wave and provides a trigger for the device [55]. Currently, the NanoKnife system delivers a pulse 50 milliseconds after each R wave [56]. Validation of trigger pulses is performed by a built-in synchronization algorithm.

Although ECG synchronization is essential, there are significant limitations that should be noted. First, it has been shown to increase the total treatment time [57] and second, synchronization relies on the occurrence of the R wave and therefore in patients who have irregular R-R intervals (e.g., atrial fibrillation), this will affect the delivery pulse frequency. Subsequently, this may produce a different effect than predicted or modeled where a consistent delivery of pulses and constant membrane effect is assumed. There is growing interest in nanosecond pulses, and translation of this safety data to nanosecond pulses is unclear and should not be assumed, and of course, this requires further evaluation.

Table 2 Electroporation: delivery and protocols

	Author	Anatomical site of ablation	Ablation lesions	Ablation device	Electric current					
				Shape of device	Pulse duration	Number of pulses	Pulse repetition frequency	Energy delivered (J, unless stated)	Peak power (V)	Peak current (A)
Atrial tissue	Lavee [39]	Atrial tissue	10	Hand held clamp	100 μs	Seq of 8, 16, 32	5 Hz	1500–2000 V	–	–
	Hong [38]	Ventricular epicardium/ atrial tissue	33	Biploar jaws Linear (suction)	100 to 400 μsec	Single train of 10–40 pulses	3–5 (1–5 hz)	–	–	–
	Wittkampf [47]	Atrial tissue	5	Circular	6 ms	5	–	200	–	–
			49	Circular 20 mm	6 ms	1	–	200	–	–
	Van Driel [45]	Pulmonary vein	10	Circular 18 mm	6 ms*	10	–	200	–	
	DeSimone [36]	Pulmonary vein		Balloon device	–	1	–	7500 uA	–	–
	Witt [51]	Pulmonary vein	10	Balloon prototype catheter	100 μs	10–200	1 Hz	1000–2000 V	–	–
Ventricular tissue	Wittkampf [46]	Ventricular epicardium	5	Circular 20 mm	6 ms	1	–	50	–	16 ± 1.3
								100	–	24.3 ± 1.3
								200	–	34.9 ± 2.1
				Circular 20 mm				100	–	25.7 ± 1.6
	Neven [41]	Ventricular epicardium	14	Linear	6 ms	1	–	30	960 ± 21	7.9 ± 0.5
								100	1845 ± 241	15.8 ± 1.2
								300	2930 ± 67	28.4 ± 1.1
	Neven [42]	Ventricular epicardium	15	Circular 12 mm	6 ms	1	–	50	1220 ± 46	11.6 ± 1.4
								100	1670 ± 74	19.0 ± 1.5
								200	2305 ± 54	27.1 ± 0.7
	Neven [43]	Ventricular epicardium	13	Circular 12 mm	6 ms*	1	–	200	–	–
	Zager [48]	Ventricular epicardium	45	2 needle electrode	100 μs	10	1 Hz	50 V	–	–
					100 μs	10	1 Hz	250 V		
					100 μs	10	1 Hz	500 V		
					70 μs	10	2 Hz	500 V		
					70 μs	10	1 Hz	500 V		
					70 μs	20	2 Hz	500 V		
	Livia [50]	Purkinje fibers (ventricular myocardium)	8	Navistar® ablation catheter	20 μs	10	1 Hz	750–2500 V	–	–
Other anatomical sites	Du Pre [37]	Coronary arteries	16	Circular 20 mm	6 ms*	1 (applied in 3 areas)	–	50–360	–	–
				Circular 20 mm				50–200	–	–
				Linear				30	–	–
	Van Driel [44]	Phrenic nerve	19	Circular 20 mm Circular 18 mm	6 ms*	1	–	200	2116 ± 152	33.0 ± 3.6
	Madhavan [40]	Cardiac ganglia plexus	27	Quadripolar ablation catheter	–	1	–	12 uA 300 or 500 uA 3000–5000 uA	–	–
			25	Deflectable multiarray	–	1	–	3000-5000 uA	–	–
	Neven [49]	Esophagus	16	Linear	6 ms	1	–	100	1737* (calculated mean)	15.5
								200	2482*	21.2

Table 3 Lesion histology characteristics

Tissue type	Author	Lesion location	Gross (macroscopic)	Histological comments	Energy (J, unless stated)	Lesion		Outcome	Collateral damage
						Width	Depth		
Ventricular tissue	Wittkampf [46]	Ventricular epicardium	- White ablation lesions - After 200 J application light purplish colorization around bruised area	Complete replacement of cardiomyocytes by granulation tissue consisting of fibroblasts with loose collagen fibers and capillaries	50 (device D)	2.6 ± 0.7	2.1 ± 0.6	0/5 lesion continuity	Nil
					100 (device D)	2.9 ± 1.2	4.5 ± 1.2	1/5 lesion continuity	
					200 (device D)	5.2 ± 1.2 (25–9-5th range 2.9 to 8.7 m-m)	5.3 ± 3.0	5/5 lesion continuity	
					100 (device M)	3.7 ± 1.2	2.8 ± 1.1	5/5 lesion continuity	
	Neven [42]	Ventricular epicardium	- Circular whitish aspect with denting in the center of the lesion - Sharp demarcation between ablation lesion and normal tissue		50 100 200	16.6 ± 1.1 18.1 ± 1.0 19.8 ± 1.8	5.0 ± 2.1 7.0 ± 2.0 11.9 ± 1.5	0% transmural 20% transmural 20% transmural	Nil
	Neven [41]	Ventricular epicardium	- Suction device caused some local epicardial hematoma - 300 J application light purplish colorization around bruised area		30 100 300	10.1 ± 0.8 15.1 ± 1.5 17.1 ± 1.3	3.2 ± 0.7 6.3 ± 1.8 8.0 ± 1.5	25% transmural 100% transmural 100% transmural	Nil
	Neven [43]	Ventricular epicardium and coronary arteries	- Whitish discoloration of ablation lesions		200		6.4 ± 2.6 mm (range, 0.0–10.4 mm)	- 4 of 13 (31%) transmural - Intimal hyperplasia in 67 or 128 coronary arteries - mean values of median luminal stenosis of the arteries showing any intimal hyperplasia were 8 ± 5%.	Nil
	Du Pre [37]	Ventricular epicardium and coronary arteries		Intimal hyperplasia in 5 or 56 arteries located inside electroporation lesion zone, with stenosis on average 22 ± 15%. None of the large coronary arteries were affected No lesion identified in LAD	30–360		6.5 ± 2.7 mm (range 1.7–13.5 mm) 2.9 ± 1.2 mm deep (range 0.2–6.3 m-m).	26 of 81 (24%) transmural.	Nil

Table 3 (continued)

Tissue type	Author	Lesion location	Gross (macroscopic)	Histological comments	Energy (J, unless stated)	Lesion		Outcome	Collateral damage
						Width	Depth		
	Zager [48]	Ventricular epicardium			Protocol 1 Protocol 2 Protocol 3 Protocol 4 Protocol 5 Protocol 6 Protocol 7		3.4 ± 5.4 8.3 ± 4.3 6.7 ± 1.8 4.4 ± 1.4 4.0 ± 0.9 5.6 ± 6.0 4.9 ± 2.9		Nil
	Hong [38]	Ventricular epicardium/ atrial tissue	No detected gross lesions	SVC and IVC lesions were 100% transmural with any/all methods of lesion evaluation. Intralesional veins and to a lesser extent arteries showed occasionally endothelial denudation	–		4 mm (RVOT, linear)		Nil
	Livia [50]	Purkinje fibers	–	–	–	–	–	–	–
Atrial Tissue	Lavee [39]	Atrial tissue	Clear demarcation line between ablation and normal tissue	Transmural lesions in 10 ablation locations.	1000 V 1500 V 2000 V	5 ± 0 5 ± 0 7.6 ± 2.5	10 ± 0 11.1 ± 2.3 7.6 ± 2.5		Nil
	Van Driel [44]	Superior vena cava (atrial tissue)	White SVC tissue at electroporation lesion.		200	Circular lesion	16.2 ± 6 mm (only done of 5 animal, average taken for all four domina-tions)	No damage to phrenic nerve. 2/19 had transient effects	Nil
	Wittkampf [46]	Superior vena cava (atrial tissue)	No gross abnormalities		200				
		Pulmonary vein		Uniform scar consisting of loose connective tissue and fibroblasts in addition to granulation tissue. Eleastic lamia was disrupted and in some cases, giant cell reaction was found.			3.5 mm (only measured in few sites)	Can create lesions and be delivered safely	
	Van Driel [45]	Pulmonary vein	PV treated by electroporation surrounded by healthy tissue	Minor intimal hyperplasia (only examined on 7 animals)	200			No PV stenosis or significant reduction in PV diameter in electroporation group.	Nil
	DeSimone [36]	Pulmonary vein	No damage to cardiac tissue	Ablation lesion extending from endocardium towards epicardium	7500 uA				Nil

Table 3 (continued)

Tissue type	Author	Lesion location	Gross (macroscopic)	Histological comments	Energy (J, unless stated)	Lesion		Outcome	Collateral damage
						Width	Depth		
	Witt [51]	Pulmonary vein	No clear ablation lesions	Transmural lesion with lesion pattern that was typically that of decellularization with only collagen scaffolding remaining. Some fibrosis observed in a minority of lesions	2000 V, 200 Pulses		3	All lesions transmural, with 5/10 (50%) lesions circumferential (ranging from 30 to 100%)	Nil
					2000 V, 100 Pulses		3		
					1000 V, 100 Pulses		5		
					1000 V, 10 Pulses		7		
					1000 V, 10 Pulses		8		
					2000 V, 200 Pulses		8		
					2000 V, 100 Pulses		15		
					2000 V, 200 Pulses		16		
					2000 V, 100 Pulses		17		
					2000 V, 200 Pulses		5		
Other Anatomical Sites	Neven [49]	Esophagus	Day 2: Normal with no stenosis, multiple whitish, circumscribed, clear fluid–containing elevations with a diameter of several millimeters, resembling vesicles in the ablated areas. 8 weeks: no macroscopically visible lesions on the adventitia or epithelium.	Day 2: Intraepithelial Vesicles. Degeneration of the superficial part of the epithelium, with intact basal epithelial layers. Lymphohistiocytic inflammatory infiltrate in the outer muscular layer with degeneration of some striated muscle cells. 8 weeks: Superficial scar was present in the outer part of the esophagus, in the outer	200			Direct electroporation ablation on the outer esophageal wall causes harmless, self-limiting vesicles on the nonkeratinizing squamous epithelium at the ablation site. After 7 and 60 days follow-up, the epithelium completely normalized. No signs of ulceration or other adverse reactions.	

Table 3 (continued)

Tissue type	Author	Lesion location	Gross (macroscopic)	Histological comments	Energy (J, unless stated)	Outcome			Collateral damage
						Lesion			
						Width	Depth		
	Madhavan [40]	Cardiac ganglia plexus	Phase 1: Phase 2: - Clusters of 3-4 mm hemorrhagic lesions at sites of ablation	part of the muscular layer. The epithelium of the mucosa was intact. Phase 1: - Ablation with loss of nucleoli - No damage to surrounding myocardium Phase 2: - Nuclear disarray and loss of cellular architecture at 17/23 sites treated	12–5000 uA				Nil

4.3 Cardiac electroporation protocols—time for standardized reporting

This review demonstrates that there is significant heterogeneity with IRE delivery tools, electroporation pulse generators, and reporting of applied electroporation parameters. The process of IRE is strongly dependent upon the pulse parameters of the delivered electric pulses and therefore to enable reproducibility, uncomplicated comparison across studies, and safe translation into human studies, the electric parameters should be described precisely [58]. Standardized terms and reporting criteria for cardiac IRE are necessary. Most studies included in this review reported the pulse length and amplitude ("energy" delivered); however, often there was a lack of reporting of other vital parameters, such as pulse frequency and a calculation of the electric field. The pulse frequency is essential as it affects temperature (with increased pulse frequency there is less time for heat dissipation between pulses) and the occurrence of muscle contraction as well as nerve stimulation [58–60]. Additionally, as shown by Zager [48], a lower frequency pulse frequency resulted in significant echocardiographic evidence of tissue damage, while the higher frequency protocols did not demonstrate any significant reduction in echocardiographic measures. Recently, recommendations for standardized reporting were published for pulsed electric field technology in food and biotechnological processes [61], life sciences/biology [62], and electrochemotherapy [63]. Based on these recommendations, we suggest that future cardiac IRE publications report the following parameters in Table 5. This formalization of reporting will not only strengthen IRE evidence-based practice and enable solid recommendations, but will also allow essential outcome comparisons with other cardiac ablative technologies.

4.4 Future developments

To date, all cardiac IRE testing has occurred in healthy animal models and there has been one human study published [32]. While this provides a solid foundation for efficacy and safety, the patients who are most likely to benefit from this new technology will have diseased hearts. The translation from a normal to a diseased model will be essential understand electroporation of cells in diseased tissues and other complex environments. This will be key to its successful use and optimization in various applications [24]. It is unclear if and what the differences that occur in the diseased myocardium will be compared to that of the normal heart. With the many variables that can alter the electric field distribution and its effectiveness, future studies should address disease models.

The results of this systematic review should provide the impetus for the development of specialized IRE delivery technology. IRE technology, however, poses novel challenges for device design. Except for a few studies, most of the IRE has

Table 4 Cardiac IRE adverse outcomes

Study	Author	Adverse outcome
1	Lavee [39]	No adverse events
2	Hong [38]	Arching observed with clamp device
3	Wittkampf [47]	No adverse events
4	Wittkampf	No adverse events
5	Du Pre [37]	One animal suffered from an episode of fever, presumably due to pericarditis.
6	Neven [41]	No adverse events
7	Neven [42]	One animal had to be euthanized acutely before electroporation applications had been delivered because of complications caused by failed subxiphoid puncture.
8	van Driel [45]	No adverse events
9	Neven [43]	One animal suddenly developed cyanosis with hemodynamic instability after the end of the index procedure, ≈ 7 h after ablation. At autopsy, no pericardial effusion or trauma other than the ablation lesions was found. Gross inspection of other organs also showed no abnormalities.
10	DeSimone [36]	No adverse events
11	Van Driel [44]	No adverse events
12	Zager [48]	Three animals died during the surgical and pre-procedural period: one during induction of anesthesia, one during traumatic intubation and one as a result of laceration of LAD during resection of the pericardium.
13	Madhavan [40]	One dog developed refractory VF during ablation at 5000 μA

Table 5 Key IRE reporting parameters

Key elements
• Electric pulse generator
○ Commercially available
▪ Company
▪ Model
○ Prototype
• Bipolar vs monopolar delivery
• Electrode material
• Electrode design
○ Shape
○ Electrode size
○ Number of electrodes
○ If more than 1 electrode, electrode spacing
• Pulse parameters
○ Number
○ Shape
○ Duration
○ Frequency (pulse repetition)
○ Voltage applied (voltage-to-distance ratio)
○ Current measured
• Electric field distribution (calculations)
• Electrode positioning with respect to target tissue

been delivered with current or slightly modified cardiac tools which were not created for the delivery of electric pulses. The currently available irrigated and non-irrigated catheters for RF energy delivery may not be ideally suited for electroporation delivery. Future devices must be compatible with catheters in a wide variety of configurations and possess steerability. Further, the electric field intensity and distribution within the tissue will vary with catheter size and electrode configuration. Specialized tools would be ideal for better ablation zone modulation and control of the electric field thereby enabling superior targeting and ultimately provide a more efficient and safe technology.

4.5 Limitations

Our study has several limitations that are common to systematic reviews. First, included studies are limited to only those that have already been published, and while a thorough effort was made with a broad search strategy, it is possible that we may have missed some relevant studies. Additionally, the studies retrieved were vastly heterogenous in IRE delivery protocols, and therefore, it is difficult (if not impossible) to draw conclusions on the optimal parameters for cardiac IRE ablation, an area that requires further work and examination and may ultimately vary depending upon the device and generator used. Second, all studies have relied on healthy models, so it is unclear at this stage the impact of IRE on disease hearts, particular from a safety and efficacy point of view.

Third, at present, there are no studies with a direct comparison with other cardiac ablation modalities which will be essential to show critical differences in this technology.

5 Conclusions

Cardiac irreversible electroporation (IRE) is an emerging ablation modality with alluring potential. This systematic review shows that IRE can be successfully and safely applied to cardiac tissue to create ablation lesions. Significant heterogeneity in the current literature raises the need to follow standard reporting of IRE parameters. This will lead to further progress in the field and improve the potential for translation into the clinical realm for human catheter ablation as we are starting to see.

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Compliance with ethical standards

Conflict of interest Authors SJA/SK/CW/CVD have filed but no issued patents within the realm of tools for electroporation. Author DM receives research funding and consulting fees from Medtronic. All other authors have no disclosures.

Ethical approval For studies by the authors as include in this review, all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

1. Edmonds JH, Ellison RG, Crews TL. Surgically induced atrioventricular block as treatment for recurrent atrial tachycardia in Wolff-Parkinson-White syndrome. Circulation. 1969;39(5S1):I-105–I-11.
2. Calkins H, Leon AR, Deam AG, Kalbfleisch SJ, Langberg JJ, Morady F. Catheter ablation of atrial flutter using radiofrequency energy. Am J Cardiol. 1994;73(5):353–6.
3. Swartz J. A catheter-based curative approach to atrial fibrillation in humans. Circulation. 1994;90:I-335.
4. Jai P, Hai M, Shah DC, Chouairi S, Gencel L, Cle J. A focal source of atrial fibrillation treated by discrete radiofrequency ablation. Circulation. 1997;95(3):572–6.
5. Haissaguerre M, Gencel L, Fischer B, Le Metayer P, Poquet F, Marcus FI, et al. Successful catheter ablation of atrial fibrillation. J Cardiovasc Electrophysiol. 1994;5(12):1045–52.
6. Hindricks G, Haverkamp W, Rissel U, Richter K, Gülker H. Experimental observations on the use of radiofrequency energy for ablation of ventricular tissue. New Trends Arrhyt. 1988;4(1–2):337–42.
7. Gonska B, Brune S, Bethge K, Kreuzer H. Radiofrequency catheter ablation in recurrent ventricular tachycardia. Eur Heart J. 1991;12(12):1257–65.
8. Nathan AW, Bennett DH, Ward DE, Bexton RS, Camm AJ. Catheter ablation of atrioventricular conduction. Lancet (London, England). 1984;1(8389):1280–4.
9. Gallagher JJ, Svenson RH, Kasell JH, German LD, Bardy GH, Broughton A, et al. Catheter technique for closed-chest ablation of the atrioventricular conduction system: a therapeutic alternative for the treatment of refractory supraventricular tachycardia. N Engl J Med. 1982;306(4):194–200.
10. Ward DE, Davies M. Transvenous high energy shock for ablating atrioventricular conduction in man. Observations on the histological effects. Heart. 1984;51(2):175–8.
11. Huang SKS. Advances in applications of radiofrequency current to catheter ablation therapy. Pacing Clin Electrophysiol. 1991;14(1):28–42.
12. Olgin JE, Scheinman MM. Comparison of high energy direct current and radiofrequency catheter ablation of the atrioventricular junction. J Am Coll Cardiol. 1993;21(3):557–64.
13. Pappone C, Oral H, Santinelli V, Vicedomini G, Lang CC, Manguso F, et al. Atrio-esophageal fistula as a complication of percutaneous transcatheter ablation of atrial fibrillation. Circulation. 2004;109(22):2724–6.
14. Black-Maier E, Pokorney SD, Barnett AS, Zeitler EP, Sun AY, Jackson KP, et al. Risk of atrioesophageal fistula formation with contact force-sensing catheters. Heart Rhythm. 2017;14(9):1328–33.
15. Sacher F, Monahan KH, Thomas SP, Davidson N, Adragao P, Sanders P, et al. Phrenic nerve injury after atrial fibrillation catheter ablation: characterization and outcome in a multicenter study. J Am Coll Cardiol. 2006;47(12):2498–503.
16. Calkins H, Reynolds MR, Spector P, Sondhi M, Xu Y, Martin A, Williams CJ, Sledge I. Treatment of atrial fibrillation with anti–arrhythmic drugs or radio frequency ablation: two systematic literature reviews and meta–analyses. Circ Arrhythm Electrophysiol. 2009:CIRCEP. 108.824789.
17. Schrickel JW, Lickfett L, Lewalter T, Mittman-Braun E, Selbach S, Strach K, et al. Incidence and predictors of silent cerebral embolism during pulmonary vein catheter ablation for atrial fibrillation. Europace. 2010;12(1):52–7.
18. Medi C, Evered L, Silbert B, Teh A, Halloran K, Morton J, et al. Subtle post-procedural cognitive dysfunction after atrial fibrillation ablation. J Am Coll Cardiol. 2013;62(6):531–9.
19. Andrade JG, Dubuc M, Guerra PG, Macle L, Mondésert B, Rivard L, et al. The biophysics and biomechanics of cryoballoon ablation. Pacing Clin Electrophysiol. 2012;35(9):1162–8.
20. Furnkranz A, Bordignon S, Bohmig M, Konstantinou A, Dugo D, Perrotta L, et al. Reduced incidence of esophageal lesions by luminal esophageal temperature-guided second-generation cryoballoon ablation. Heart Rhythm. 2015;12(2):268–74.
21. Narui R, Tokuda M, Matsushima M, Isogai R, Tokutake K, Yokoyama K, et al. Incidence and factors associated with the occurrence of pulmonary vein narrowing after cryoballoon ablation. Circ Arrhythm Electrophysiol. 2017;10(6).
22. Ichihara N, Miyazaki S, Iwasawa J, Matsuda J, Taniguchi H, Nakamura H, et al. Prevalence and pre-procedural predictors associated with right phrenic nerve injury in electromyography-guided, second-generation cryoballoon ablation. JACC Clin Electrophysiol. 2016;2(4):508–14.
23. Aksu T, Ebru Golcuk S, Yalin K. Haemoptysis and pulmonary haemorrhage associated with cryoballoon ablation. Europace. 2015;17(8):1240.
24. Rems L, Miklavčič D. Tutorial: electroporation of cells in complex materials and tissue. J Appl Phys. 2016;119(20):201101.

25. Martin RC, McFarland K, Ellis S, Velanovich V. Irreversible electroporation in locally advanced pancreatic cancer: potential improved overall survival. Ann Surg Oncol. 2013;20(3):443–9.
26. Paiella S, Butturini G, Frigerio I, Salvia R, Armatura G, Bacchion M, et al. Safety and feasibility of irreversible electroporation (IRE) in patients with locally advanced pancreatic cancer: results of a prospective study. Dig Surg. 2015;32(2):90–7.
27. Valerio M, Stricker PD, Ahmed HU, Dickinson L, Ponsky L, Shnier R, et al. Initial assessment of safety and clinical feasibility of irreversible electroporation in the focal treatment of prostate cancer. Prostate Cancer Prostatic Dis. 2014;17(4):343–7.
28. Scheffer HJ, Nielsen K, de Jong MC, van Tilborg AA, Vieveen JM, Bouwman AR, et al. Irreversible electroporation for nonthermal tumor ablation in the clinical setting: a systematic review of safety and efficacy. J Vasc Interv Radiol. 2014;25(7):997–1011.
29. Welden CV, Christein JD, Wilcox CM, Ahmed AM. Initial experience of irreversible electroporation in the treatment of locally advanced pancreatic adenocarcinoma. Gastroenterology. 2017;152(5):S277.
30. Wojtaszczyk A, Caluori G, Pesl M, Melajova K, Starek Z. Irreversible electroporation ablation for atrial fibrillation. J Cardiovasc Electrophysiol. 2018;29(4):643–51.
31. Sugrue A, Maor E, Ivorra A, Vaidya V, Witt C, Kapa S, et al. Irreversible electroporation for the treatment of cardiac arrhythmias. Expert Rev Cardiovasc Ther. 2018;16(5):349–60.
32. Reddy VY, Koruth J, Jais P, Petru J, Timko F, Skalsky I, et al. Ablation of atrial fibrillation with pulsed electric fields: an ultra-rapid, tissue-selective modality for cardiac ablation. JACC Clin Electrophysiol. 2018:674.
33. Vries R, Hooijmans CR, Langendam MW, Luijk J, Leenaars M, Ritskes-Hoitinga M, et al. A protocol format for the preparation, registration and publication of systematic reviews of animal intervention studies. Evid Based Preclin Med. 2015;2(1):1–9.
34. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097.
35. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol. 2010;8(6):e1000412.
36. DeSimone CV, Ebrille E, Syed FF, Mikell SB, Suddendorf SH, Wahnschaffe D, 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(6):508–14.
37. du Pre BC, van Driel VJ, van Wessel H, Loh P, Doevendans PA, Goldschmeding R, et al. Minimal coronary artery damage by myocardial electroporation ablation. Europace. 2013;15(1):144–9.
38. Hong J, Stewart MT, Cheek DS, Francischelli DE, Kirchhof N. Cardiac ablation via electroporation. Conf Proc IEEE Eng Med Biol Soc. 2009;2009:3381–4.
39. Lavee J, Onik G, Mikus P, Rubinsky B, editors. A novel nonthermal energy source for surgical epicardial atrial ablation: irreversible electroporation. Heart Surgery Forum; 2007: FORUM MULTIMEDIA PUBLISHING.
40. Madhavan M, Venkatachalam K, Swale MJ, Desimone CV, Gard JJ, Johnson SB, et al. Novel percutaneous epicardial autonomic modulation in the canine for atrial fibrillation: results of an efficacy and safety study. Pacing Clin Electrophysiol. 2016;39(5):407–17.
41. Neven K, van Driel V, van Wessel H, van Es R, Doevendans PA, Wittkampf F. Epicardial linear electroporation ablation and lesion size. Heart Rhythm. 2014;11(8):1465–70.
42. Neven K, van Driel V, van Wessel H, van Es R, Doevendans PA, Wittkampf F. Myocardial lesion size after epicardial electroporation catheter ablation following subxiphoid puncture. Circ Arrhythm Electrophysiol. 2014:CIRCEP. 114.001659.
43. Neven K, van Driel V, van Wessel H, van Es R, du Pre B, Doevendans PA, et al. Safety and feasibility of closed chest epicardial catheter ablation using electroporation. Circ Arrhythm Electrophysiol. 2014;7(5):913–9.
44. van Driel VJ, Neven K, van Wessel H, Vink A, Doevendans PA, Wittkampf FH. Low vulnerability of the right phrenic nerve to electroporation ablation. Heart Rhythm. 2015;12(8):1838–44.
45. van Driel VJ, Neven KG, van Wessel H, du Pre BC, Vink A, Doevendans PA, et al. Pulmonary vein stenosis after catheter ablation: electroporation versus radiofrequency. Circ Arrhythm Electrophysiol. 2014;7(4):734–8.
46. Wittkampf FH, van Driel VJ, van Wessel H, Neven KG, Grundeman PF, Vink A, et al. Myocardial lesion depth with circular electroporation ablation. Circ Arrhythm Electrophysiol. 2012;5(3):581–6.
47. Wittkampf FH, van Driel VJ, van Wessel H, Vink A, Hof IE, Grundeman PF, et al. Feasibility of electroporation for the creation of pulmonary vein ostial lesions. J Cardiovasc Electrophysiol. 2011;22(3):302–9.
48. Zager Y, Kain D, Landa N, Leor J, Maor E. Optimization of irreversible electroporation protocols for in-vivo myocardial decellularization. PLoS One. 2016;11(11):e0165475. PMCID: PMC5125564 a patent application entitled "Myocardial Ablation by Irreversible Electroporation" (Application #: US14/894,349). This patent relates in part to the results presented in this study. In addition, Dr. Maor has a granted patent entitled "Extracellular matrix material created using non-thermal irreversible electroporation" (US8835166 B2). There are no additional patents, products in development or marketed products to declare. This does not alter our adherence to all the PLOS ONE policies on sharing data and materials, as detailed online in the guide for authors.
49. Neven K, van Es R, van Driel V, van Wessel H, Fidder H, Vink A, et al. Acute and long-term effects of full-power electroporation ablation directly on the porcine esophagus. Circ Arrhythm Electrophysiol. 2017;10(5):e004672.
50. Livia C, Sugrue A, Witt T, Polkinghorne MD, Maor E, Kapa S, et al. Elimination of Purkinje fibers by electroporation reduces ventricular fibrillation vulnerability. J Am Heart Assoc. 2018;7(15):e009070.
51. Witt CM, Sugrue A, Padmanabhan D, Vaidya V, Gruba S, Rohl J, et al. Intrapulmonary vein ablation without stenosis: a novel balloon-based direct current electroporation approach. J Am Heart Assoc. 2018;7(14):e009575.
52. Thomson KR, Cheung W, Ellis SJ, Federman D, Kavnoudias H, Loader-Oliver D, et al. Investigation of the safety of irreversible electroporation in humans. J Vasc Interv Radiol. 2011;22(5):611–21.
53. Charpentier KP, Wolf F, Noble L, Winn B, Resnick M, Dupuy DE. Irreversible electroporation of the liver and liver hilum in swine. HPB. 2011;13(3):168–73.
54. Cannon R, Ellis S, Hayes D, Narayanan G, Martin RC 2nd. Safety and early efficacy of irreversible electroporation for hepatic tumors in proximity to vital structures. J Surg Oncol. 2013;107(5):544–9.
55. Mali B, Gorjup V, Edhemovic I, Brecelj E, Cemazar M, Sersa G, et al. Electrochemotherapy of colorectal liver metastases-an observational study of its effects on the electrocardiogram. Biomed Eng Online. 2015;14(3):S5.
56. Bertacchini C, Margotti PM, Bergamini E, Lodi A, Ronchetti M, Cadossi R. Design of an irreversible electroporation system for clinical use. Technol Cancer Res Treat. 2007;6(4):313–20.
57. Deodhar A, Dickfeld T, Single GW, Hamilton WC Jr, Thornton RH, Sofocleous CT, et al. Irreversible electroporation near the heart: ventricular arrhythmias can be prevented with ECG synchronization. Am J Roentgenol. 2011;196(3):W330–W5.
58. Reberšek M. Beyond electroporation pulse parameters: from application to evaluation. Handb Electroporation. 2017:1–21.

59. Schoenbach KH. From the basic science of biological effects of ultrashort electrical pulses to medical therapies. Bioelectromagnetics. 2018;39:257–76.
60. Mercadal B, Arena CB, Davalos RV, Ivorra A. Avoiding nerve stimulation in irreversible electroporation: a numerical modeling study. Phys Med Biol. 2017;62(20):8060–79.
61. Raso J, Frey W, Ferrari G, Pataro G, Knorr D, Teissie J, et al. Recommendations guidelines on the key information to be reported in studies of application of PEF technology in food and biotechnological processes. Innovative Food Sci Emerg Technol. 2016;37:312–21.
62. 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.
63. Campana LG, Clover AJ, Valpione S, Quaglino P, Gehl J, Kunte C, et al. Recommendations for improving the quality of reporting clinical electrochemotherapy studies based on qualitative systematic review. Radiol Oncol. 2016;50(1):1–13.