JACC: CLINICAL ELECTROPHYSIOLOGY VOL. ■, NO. ■, 2021

PUBLISHED BY ELSEVIER

INNOVATIONS IN BASIC/TRANSLATIONAL ELECTROPHYSIOLOGY

Comparing High-Frequency With Monophasic Electroporation Protocols in an In Vivo Beating Heart Model

Eyal Heller, MD, BSc,[a,b,*] Tomas Garcia-Sanchez, PhD,[c,*] Yonatan Moshkovits, BSc,[a,b] Raul Rabinovici, PhD,[d] Dvora Grynberg, BSc,[a,b] Amit Segev, MD,[a,b,d] Samuel J. Asirvatham, MD,[e] Antoni Ivorra, PhD,[c,f] Elad Maor, MD, PhD[a,b]

ABSTRACT

This study compared monophasic 100-μs pulses with high-frequency electroporation (HF-EP) bursts using an in vivo animal model. Myocardial damage was evaluated by histologic analysis. Compared with 10 monophasic pulses, 20 bursts of HF-EP at 100 and 150 kHz were associated with less damage. However, when the number of HF-EP bursts was increased to 60, myocardial damage was comparable to that of the monophasic group. HF-EP protocols were associated with attenuated collateral muscle contractions. This study shows that HF-EP is feasible and effective and that pulse frequency has a significant effect on extent of ablation. (J Am Coll Cardiol EP 2021;■:■–■) © 2021 by the American College of Cardiology Foundation.

Recent preclinical and first-in-human data confirm that irreversible electroporation (IRE), also termed pulsed field ablation (PFA), might be both efficient and safe for the treatment of cardiac arrhythmias (1). Contemporary electroporation data are based on experiments by several groups, differing in electroporation protocols and electrode configurations (2,3). Unfortunately, electroporation protocol specifications are not disclosed by the industry. Although traditional IRE protocols use sequences of square 100-μs monophasic pulses, these protocols cause nerve stimulation, resulting in muscle contractions requiring muscle relaxants, anesthesia, or sedation (4). High-frequency IRE protocols (termed H-FIRE) consisting of bursts of square biphasic pulses of short duration (<10 μs/phase) have demonstrated a reduction or complete elimination of muscle contractions in noncardiac tissue (2,5). Therefore, this study aimed to compare the traditional IRE protocol, consisting of monophasic (i.e., direct current [DC]) 100-μs pulses, with a high-frequency electroporation (HF-EP) protocol in an in vivo beating heart animal model.

METHODS

The characteristics of the high-frequency protocols used in this study are similar to the H-FIRE protocols previously proposed for noncardiac tissues (2,5). The main difference of the protocols proposed in the present study is that the delay between the positive and negative phases of the biphasic pulses was set to

From the [a]Neufeld and Tamman Cardiovascular Research Institutes, Sheba Medical Center, Sackler School of Medicine, Tel Aviv University, Israel; [b]Heart Center, Sheba Medical Center, Tel Hashomer, Israel; [c]Department of Information and Communication Technologies, Universitat Pompeu Fabra, Barcelona, Spain; [d]Department of Electrical and Computer Engineering, Ben-Gurion University of the Negev, Beer-Sheva, Israel; [e]Department of Cardiovascular Medicine, Mayo Clinic, Rochester, Minnesota, USA; and the [f]Serra Húnter Fellow Programme, Universitat Pompeu Fabra, Barcelona, Spain. *Drs Heller and Garcia-Sanchez contributed equally to this work.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Manuscript received April 2, 2021; revised manuscript received May 7, 2021, accepted May 8, 2021.

ISSN 2405-500X/$36.00 https://doi.org/10.1016/j.jacep.2021.05.003

ABBREVIATIONS AND ACRONYMS

DC = direct current

H-FIRE = high-frequency irreversible electroporation

HF-EP = high-frequency electroporation

IRE = irreversible electroporation

PFA = pulsed field ablation

0. This modification was motivated by recent studies that used sine waves in electroporation treatments (6) and, in particular, demonstrated their feasibility to create irreversible lesions in liver tissue while avoiding skeletal muscle stimulation (7). This study included 4 different protocols: Protocol 1 is the traditional IRE protocol consisting of 10 monophasic DC square pulses of 100-μs duration at a repetition rate of 1 pulse per second and is the control group for this study. Protocols 2 through 4 are HF-EP protocols using biphasic square pulses, applied in bursts of 100-μs duration at a repetition rate of 1 burst per second. They differ from each other in either the frequency of the square wave or the number of bursts: protocols 2 and 3 have the same number of bursts (n = 20) but different frequencies (100 kHz vs 150 kHz); protocols 3 and 4 have the same frequency (150 kHz) but different numbers of bursts (n = 20 vs 60). (see table in **Central Illustration**). In all protocols, pulses/bursts were applied at a repetition frequency of 1 pulse/burst per second, with a peak amplitude of 550 V.

EQUIPMENT

We assembled a custom-made generator for HF-EP and used a BTX ECM 830 (Harvard Apparatus) generator for standard electroporation. Both the BTX and the high-frequency, high-voltage pulse generator were connected to 2 needle electrodes, each 3 mm long and with a 5-mm distance between them.

COMPUTER SIMULATIONS

The main goal of these simulations was to describe electric field distribution and to ensure no temperature increase. The model was built using COMSOL Multiphysics 5.3 and simultaneously solved the electric potential, the temperature, and the electrical conductivity distributions in the treatment region during pulse application. Only the temperature dynamics was solved in the period between pulses. This 2-step process was sequentially repeated N times, N being the number of pulses/bursts applied, updating in each step the initial values from the previous iteration. The heat source in the system corresponds to the joule heating produced by the passage of an electric current through a conductor ($\vec{J} \cdot \vec{E}$), where $\vec{E}$ is the electric field, and $\vec{J}$ is the current density ($\vec{J} = \sigma \vec{E}$). To account for the electroporation phenomenon, the conductivity σ of the heart tissue was defined as a sigmoid function of the local electric field magnitude, $\left|\vec{E}\right|$. See **Figure 1**.

ANIMAL MODEL

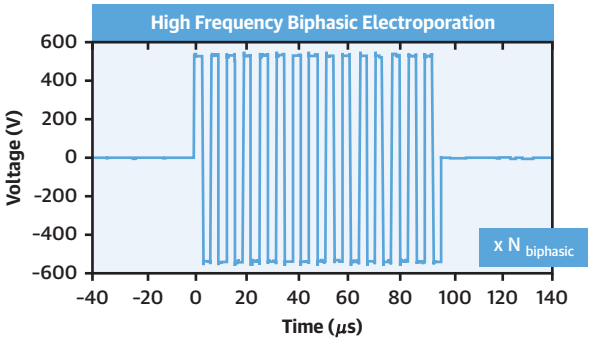
This study was approved by the ethical committee of the Chaim Sheba Medical Center (approval number 1171-18-ANIM). Sprague-Dawley rats were used in this study. Under sterile conditions and as previously described (8), PFA was then applied to the rat's beating heart through needle electrodes. The occurrence of muscle contractions during the application of pulses/bursts was documented by video. Animals were sacrificed after 14 days of follow-up. Heart specimens were stained using hematoxylin and eosin for morphometric measurements and with Picrosirius stains for the evaluation of the degree of fibrosis. Morphometric measurements (perimeter and thickness) were done using CellSense Imagine software (Olympus), and fibrosis was measured using Fiji software (ImageJ). The degree of damage was evaluated using these 3 parameters: thickness ratio, perimeter ratio, and degree of fibrosis. First, the thickness of the thinnest area of each scar and the thickness of a healthy area in the same slide were measured, and their ratio was calculated. Second, the scarred tissue portion of the total perimeter of the left ventricle was calculated and presented as a percentage ratio. Third, the percentages of fibrotic areas in both healthy and damaged myocardium were calculated and were used to calculate the ratio of fibrosis between damaged and healthy myocardium. Protocols were compared by unpaired Student's *t*-test. Statistical significance was defined as $P < 0.05$.

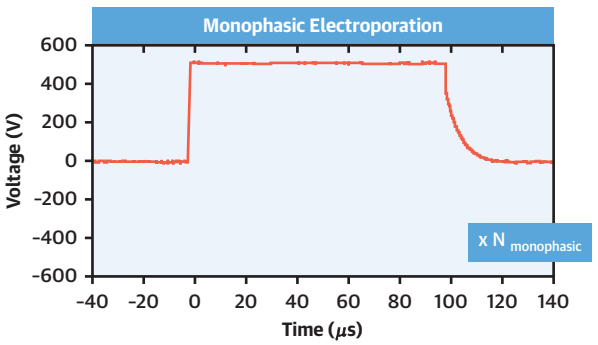
RESULTS

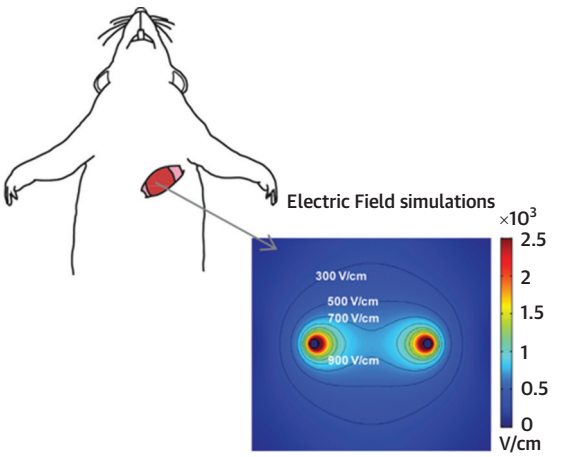
COMPUTER SIMULATIONS. Consistent with previous observations (8), the electric field distribution between needle pairs is highly inhomogeneous, with high-intensity fields around the needles that rapidly decrease through the center of the domain. However, as electroporation is a threshold phenomenon (dead above the threshold, alive below the threshold), this inhomogeneity will not become apparent in the IRE outcome. The simulations show that the tissue in the central region between the needles will be damaged if the electric field is above an electric field ranging from 500 to 700 V/cm. The results ensured there was no thermal damage to the heart tissue during the experiments, with a maximum accumulated temperature increase of less than 3 °C.

EXPERIMENTAL OBSERVATIONS. Thirty animals were included in the final analysis. No differences in immediate survival were noticed between study groups, and mortality was attributed to the surgical

CENTRAL ILLUSTRATION **Pulsed Field Ablation With High-Frequency Irreversible Electroporation**







Protocols

Protocol	Frequency	N of Pulses/ Bursts	Pulse Amplitude	Pulse/Burst Duration
1	Direct Current (monophasic)	10	550 V	100 μs
2	100 kHz	20	550 V	100 μs
3	150 kHz	20	550 V	100 μs
4	150 kHz	60	550 V	100 μs

Heller, E. et al. J Am Coll Cardiol EP. 2021;■(■):■–■.

The main point of the paper is demonstrated in this illustration. Bipolar needle configuration delivered high-voltage, high-frequency pulses to a rodent beating heart in 3 different protocols. The damage of each of these 3 protocols was compared to that of a traditional monophasic electroporation protocol.

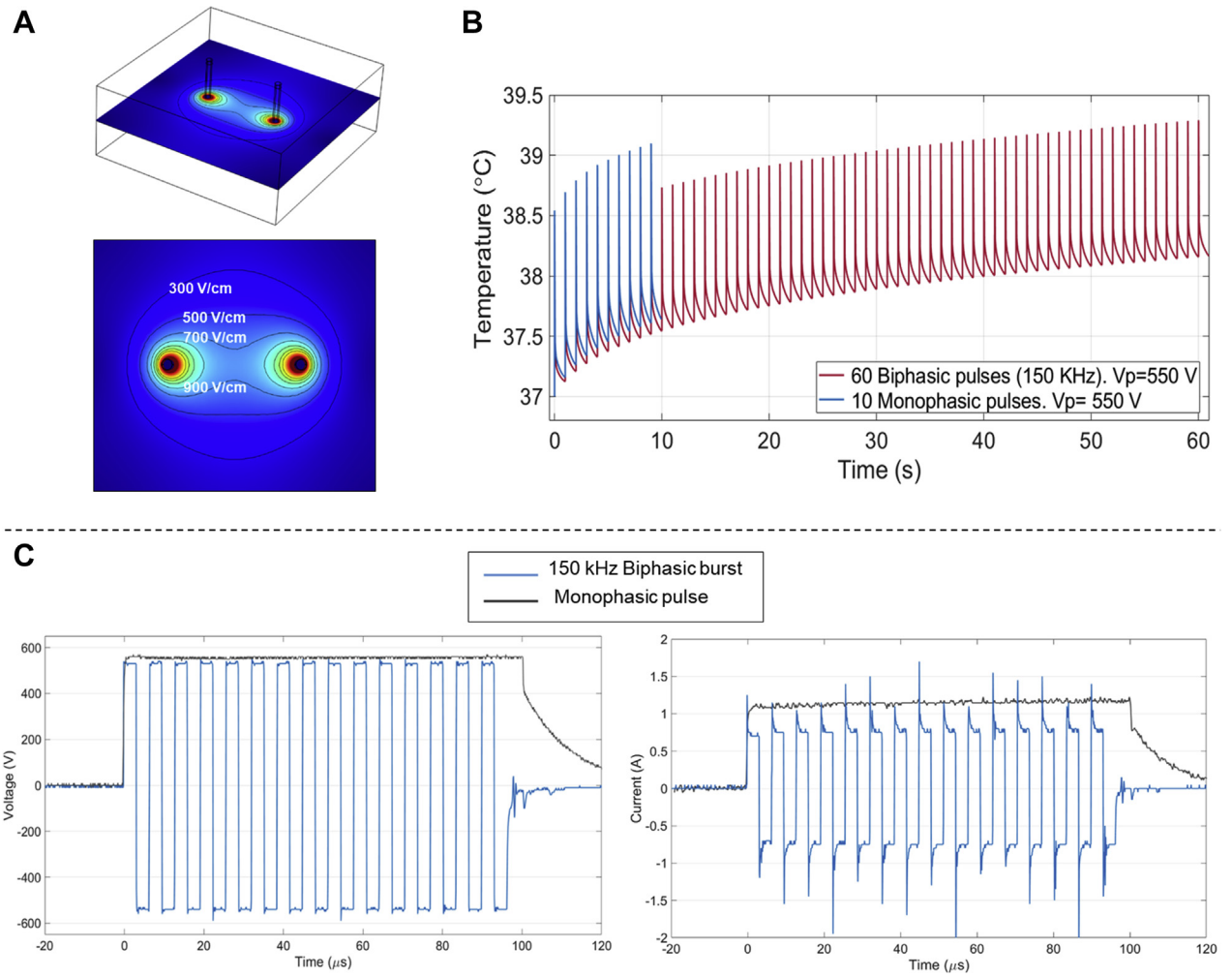
procedure. All 30 animals survived the 14 days of follow-up without any adverse events. There were 6, 9, 8, and 7 animals in protocol groups 1, 2, 3, and 4, respectively. Examples of voltage and current recordings during the first pulse/burst of a treatment with conventional monophasic pulses and with the high-frequency biphasic square waveforms are shown in Figure 1. An increase of current during the course of this first burst was systematically observed for both the 150-kHz high-frequency (mean increase of 9.4 ± 3.1%) and monophasic pulses (mean increase of 10.1 ± 2%). However, following the first pulse, subsequent pulses showed no increase in mean current in both the monophasic and high-frequency protocols. In contrast to the monophasic pulses, no significant contractions were observed with any of the HF-EP protocols (Figure 1, Video 1). No qualitative differences were noticed between contractions in the 100 kHz and 150 kHz groups.

HISTOLOGIC OBSERVATIONS. Both monophasic and HF-EP pulses yielded consistent results with respect to the overall histologic appearance of the ablated tissue (Figure 2). Scarred tissue of all protocols demonstrated marked fibrosis and a thin-appearing scar, with no evidence of normal myocardium within the ablated zone and with a clear demarcation line between healthy and ablated myocardium.

Effects of the monophasic electroporation protocol. Compared with healthy untreated myocardium, ablated myocardium was associated with 28-fold increase in the percentage of fibrosis (0.7 ± 0.3% vs 20 ± 10%, respectively; $P < 0.01$). With respect to the total perimeter of the myocardial tissue, monophasic electroporation induced damage to 25% of the total perimeter (5.4 ± 2.6 mm of the total perimeter of 21.4 ± 5.3 mm) and thinning of the myocardium by 23% (918 ± 502 μm vs 213 ±198 μm, respectively; $P < 0.01$) (Figure 2).

Effect of frequency. Protocols 2 and 3 differed only in the frequency of the applied bursts with 100 kHz and 150 kHz, respectively. In terms of fibrosis, both protocols had significantly less damage than in protocol 1. Protocol 2 displays a ratio of fibrosis between treated and healthy tissue of 12.8 ± 9.4% and protocol

FIGURE 1 **Simulation Results and Pulse Measurements**

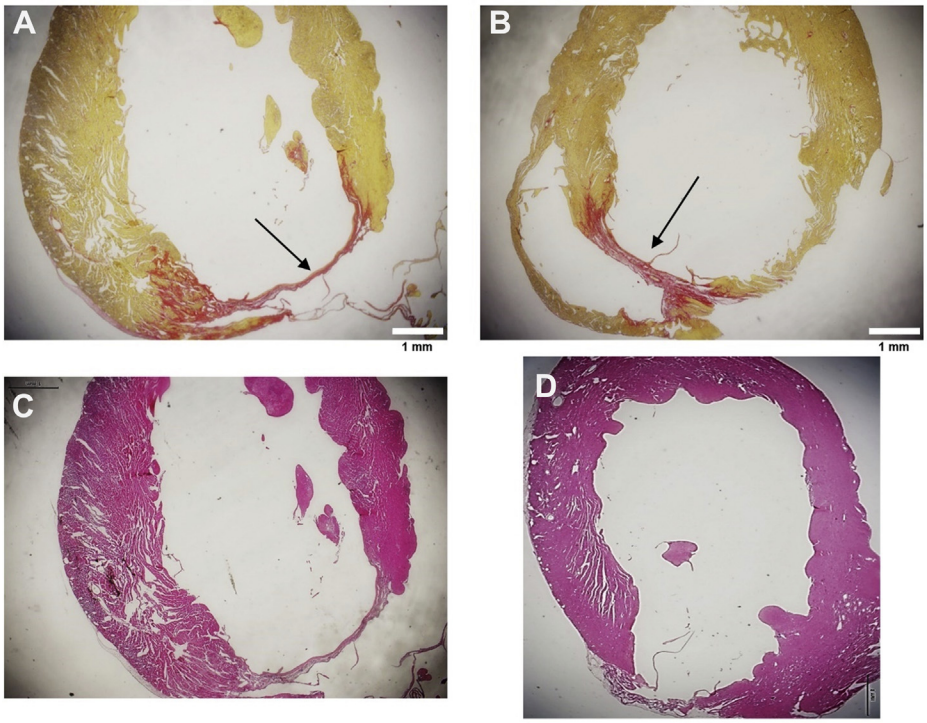


(A) Electric field intensity distribution. Contour lines depict regions with the same electric field intensity. **(B)** Evolution of maximum temperature in the 2 conditions studied (60 biphasic 150-kHz bursts or 10 monophasic pulses). **(C, D)** Recordings of **(C)** voltage and **(D)** current for a 150-kHz burst **(blue)** and a monophasic pulse **(black)** during delivery to the myocardium through the 2 needle electrodes. The average current peak of the HF-EP pulse is approximately 0.8 A, whereas the average current of the monophasic pulse is 1.1 A. Note the slight increment in the current of both protocols during the 100-μs pulse/burst. The actual delivered burst durations were 96.35 ± 3.8 μs.

3, 7.4 ± 2.1%, whereas this ratio in protocol 1 is 27.5 ± 11.2%. This suggests a reduced efficiency of these pulsing conditions. In contrast, when comparing thickness and perimeter ratios, protocol 2 was comparable to protocol 1 (26.9 ± 7% vs 21.3 ± 8.3%; $P = 0.19$ and 17.1 ± 4.1% vs 25.1 ± 12.6 %; $P = 0.13$, respectively), whereas protocol 3 still has significant differences in these parameters compared to monophasic pulses (37.9 ± 11.1% and 13.5 ± 5.8%; $P < 0.01$ and $P = 0.01$, respectively). These results suggest that for the same number of bursts applied, the lowest frequency (100 kHz) was more efficient.

Effect of number of bursts. Protocols 3 and 4 both used a frequency of 150 kHz but differed in the number of bursts applied (N = 20 vs 60 pulses, respectively). When increasing the number of bursts to 60, tissue damage was comparable to that of the monophasic pulses, with nonsignificant differences in any of the evaluated parameters (fibrosis ratio: 19.4 ± 4.2, $P = 0.10$; thickness ratio: 32.9 ± 19.3%, $P = 0.14$;

FIGURE 2 **Histology After Monophasic and High-Frequency Electroporation Treatment**



Histology of myocardial tissue after anterior myocardium was treated with **(A,C)** monopolar electroporation pulses and **(B,D)** 60 pulses of high-frequency electroporation at a frequency of 150 kHz. **(A, B)** Picrosirius stain for the degree of fibrosis **(red area** indicates fibrin for the damaged tissue). **(C, D)** Hematoxylin and eosin stain.

perimeter ratio: 19.4 ± 8.1%, $P = 0.30$). An increase in the number of bursts demonstrated a significant increase in terms of fibrosis ($P < 0.01$) but no significant differences in terms of thickness and perimeter ($P = 0.52$ and $P = 0.11$, respectively).

DISCUSSION

The main finding of the current study is that HF-EP is efficient and induces comparable damage to that of standard DC electroporation with attenuated collateral muscle excitation. Similar to previous studies that applied HF-EP bursts to various organs (2,7), attenuated muscle contractions were seen in our study when biphasic square pulses of either 100 or 150 kHz were used. Our results of attenuated efficacy at high frequencies are supported by previous observations in noncardiac tissues (7). Electroporation protocols are not fully disclosed by the industry. Most recently, Reddy et al (1) used PFA for ablation of atrial fibrillation. Although neither the frequency nor the duration of the bursts was disclosed, protocols were described as optimized biphasic waveforms. The extent of muscle contractions was not quantified in their studies. In the studies by Stewart et al (9), a protocol of trains of biphasic cycles with a phase duration of 100 μs and a cycle duration of approximately 600 μs was used. In this case, each burst had a long duration of 36 ms. The study again yielded satisfactory results in a porcine model (9), yet it did not quantify the extent of muscle contractions and used pharmacologic sedation. Loh et al (10) have successfully performed first-in-human bidirectional pulmonary veins isolation using a single millisecond-duration DC pulse. During the procedure, patients were under general anesthesia, and no documentation of muscle contractions was provided (2).

FUNDING SUPPORT AND AUTHOR DISCLOSURES

Funded by the Nicholas and Elizabeth Shlezak Super Center for Cardiac Research and Biomedical Engineering at Tel Aviv University; Mayo Clinic-Sheba innovation grant; project (PID2019-110120RBI00/AEI/10.13039/501100011033) from the Ministry of Science, Innovation, and Universities; and the State Research Agency of the Spanish government and by the Beatriu de Pinos program from the Ministry of Business and Knowledge of the Government of Catalonia (grant

identifier: 2017 BP 00032). Dr Ivorra gratefully acknowledges financial support by the Catalan Institution for Research and Advanced Studies (ICREA) under the ICREA Academia program. Dr Garcia-Sanchez is a consultant for Argá Medtech SA. Dr Ivorra is a consultant for Argá Medtech SA. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr Elad Maor, Leviev Heart Center, Sheba Medical Center, Tel Hashomer, Derech Sheba 2, Ramat Gan, Israel. E-mail: Elad.Maor@Sheba.health.gov.il.

REFERENCES

1. Reddy VY, Anic A, Koruth J, et al. Pulsed field ablation in patients with persistent atrial fibrillation. *J Am Coll Cardiol*. 2020;76(9):1068–80.

2. Arena CB, Sano MB, Rossmeisl JH, et al. High-frequency irreversible electroporation (H-FIRE) for non-thermal ablation without muscle contraction. *Biomed Eng Online*. 2011;10:102.

3. Sugrue A, Vaidya VR, Livia C, et al. Feasibility of selective cardiac ventricular electroporation. *PLoS One*. 2020;15(2).

4. Rogers WR, Merritt JH, Comeaux JA, et al. Strength-duration curve an electrically excitable tissue extended down to near 1 nanosecond. *IEEE Trans Plasma Sci*. 2004;32(4 II):1587–99.

5. Miklovic T, Latouche EL, DeWitt MR, Davalos RV, Sano MB. A comprehensive characterization of parameters affecting high-frequency irreversible electroporation lesions. *Ann Biomed Eng*. 2017;45(11):2524–34.

6. Garcia-Sanchez T, Mercadal B, Polrot M, et al. Successful tumor electrochemotherapy using sine waves. *IEEE Trans Biomed Eng*. 2020;67(4):1040–9.

7. Castellví Q, Mercadal B, Moll X, Fondevila D, Andaluz A, Ivorra A. Avoiding neuromuscular stimulation in liver irreversible electroporation using radiofrequency electric fields. *Phys Med Biol*. 2018;63(3):035027.

8. 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. https://doi.org/10.1371/journal.pone.0165475.

9. Stewart MT, Haines DE, Verma A, et al. Intracardiac pulsed field ablation: proof of feasibility in a chronic porcine model. *Heart Rhythm*. 2018;16(5):P754–64.

10. Loh P, van Es R, Groen MHA, et al. Pulmonary vein isolation with single pulse irreversible electroporation: a first in human study in 10 patients with atrial fibrillation. *Circ Arrhythm Electrophysiol*. 2020;13(10):e008192.

KEY WORDS **H-FIRE, electroporation, muscle contraction, pulsed field ablation**

APPENDIX **For a supplemental video, please see the online version of this paper.**