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Significant hemolysis is present during irreversible electroporation of cardiomyocytes in vitro

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ABSTRACT

BACKGROUND Pulsed field ablation (PFA) of atrial fibrillation is a new method in clinical practice. Despite a favorable safety profile of PFA in atrial fibrillation ablation, rare cases of renal failure, probably due to hemolysis, have recently been reported.

OBJECTIVE The aim of this study was to determine the rate of hemolysis and cardiac cell death during in vitro PFA with different electric field intensities.

METHODS Blood samples from healthy volunteers and mouse HL-1 cardiomyocyte cell lines were subjected to in vitro irreversible electroporation using 216 bipolar pulses, each lasting 2 μs with intervals of 5 μs, repeated 20 times at a frequency of 1 Hz. These pulses varied from 500 V to 1500 V. Cell-free hemoglobin levels were assessed spectrophotometrically, and red blood cell microparticles were evaluated by flow cytometry. Cardiomyocyte death was quantified with propidium iodide.

RESULTS Pulsed field energy (1000 V/cm, 1250 V/cm, and 1500 V/cm) was associated with a significant increase in cell-free hemoglobin (0.32 ± 0.16 g/L, 2.2 ± 0.96 g/L, and 5.7 ± 0.39 g/L; $P < .01$) and similar increase in the concentration of red blood cell microparticles. Significant rates of cardiomyocyte death were observed at electric field strengths of 750 V/cm, 1000 V/cm, 1250 V/cm, and 1500 V/cm (26.5% ± 5.9%, 44.3% ± 6.2%, 55.5% ± 6.9%, and 74.5% ± 17.8% of cardiomyocytes; $P < .01$).

CONCLUSION The most effective induction of cell death in vitro was observed at 1500 V/cm. This intensity was also associated with a significant degree of hemolysis.

KEYWORDS Atrial fibrillation; Cardiomyocytes; Hemolysis; Irreversible electroporation; Pulsed field ablation

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Introduction

Pulsed field ablation (PFA), or irreversible electroporation (IRE), presents a new nonthermal energy that is increasingly used for catheter ablation of atrial fibrillation.[1] In preclinical studies, PFA was associated with high myocardial selectivity and preservation of surrounding nonmyocardial structures. Accordingly, no cases of pulmonary vein stenosis or atrial-esophageal fistula have been reported in clinical practice.[1] Targeted myocardial damage and retention of nonmyocardial atrial stroma also contribute to the preservation of nerve tissue and blood vessels in the atrial wall.

The principle of PFA is to produce irreversible pores in the cytoplasmic membrane by high-energy electric pulses of short duration and high frequency.[2] These pores allow the free passage of ions.[3] Given that the extracellular concentration of Ca^{2+} is notably higher than the intracellular concentration, Ca^{2+} flows down its concentration gradient into the cell, causing an intracellular overload of Ca^{2+}, Ca^{2+}-related disruption of various metabolic pathways, and ultimately cell death.[4]

Although the method has been thoroughly studied, instances of acute renal failure have been attributed to

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procedural hemolysis, necessitating temporary hemodialysis in some cases.[5] Numerous in vitro[6–9] and in vivo[10,11] studies have examined the impact of various electroporation protocols commonly used for IRE on cardiomyocytes, nonmyocardial cardiac tissue, and surrounding structures, such as pulmonary veins, the aorta, and the esophagus. Interestingly, the effect of PFA on erythrocytes was not described in these studies, although the effect of isolated high-voltage pulse on erythrocytes was described as early as the 1970s.

Our in vitro pulsed field (PF) system allows set up of electroporation parameters, including electric field strength, pulse length, pulse polarity, and pulse number of repetition. Our study aimed to evaluate the impact of PFA on hemolysis and cardiomyocyte cell death under in vitro conditions. We sought to determine how erythrocyte and cardiomyocyte injury varies with different electric field strengths.

Methods

Electric field modeling

Because it is not possible to directly measure the high electric fields achieved and the value in tissue depends not only on applied voltage but also on electrode shape, distance between electrodes, and electrical conductivity of particular tissue, numerical simulation had to be performed. Details of the simulation and parameters are shown in the Supplemental Methods. and in Supplemental Tables S1 and S2 and Supplemental Figures S1–S3.

Ethics statement and obtaining blood samples

Blood samples were obtained from healthy volunteers according to the European Union regulation on collecting and using human body materials for research purposes. Both sexes were included in the study. All blood samples were obtained while patients were fasting. Samples were drawn from antecubital veins and without tourniquets; the first 5 mL of blood was discarded. Hemoglobin concentration was assessed in all volunteers, and all were within the normal range. The study was approved by the Ethics Committee of the University Hospital Kralovske Vinohrady (EK-VP/58l0l2023) and was conducted according to the Declaration of Helsinki. All healthy volunteers signed an informed consent document before blood donation.

Cell culture

For cell experiments, the immortalized cell line of HL-1 cardiomyocytes (SCC065; Merck, St Louis, MO) was used. Cells were cultured in Claycomb medium (51800C; Merck) according to an optimized protocol provided by Dr Claycomb's laboratory.[12] For all experiments, passages 9–12 were used, all with at least 80% confluency (approximately 4 days after seeding). For electroporation in 96-well plates (Thermo Fisher, Waltham, MA), cells were seeded at a density of 35,000 cells/well. All experiments were performed at 100% cell confluence.

Abbreviations

IRE: irreversible electroporation

PF: pulsed field

PFA: pulsed field ablation

RBC: red blood cell

RBCμ: red blood cell microparticle

RMSE: root mean square error

In vitro IRE protocol

PFA was conducted with a commercial Tonapulse (Tonagena, Kladno, Czech Republic) electrical pulse generator with an electrode plate (Figure 1A and 1B). A detailed description of the electrodes is given in the Supplemental Methods. A model of electrode placement in a single well is shown in Figure 1C.

For the blood experiments, samples of whole blood were collected in the 10-mL sodium citrate vacutainers. Blood samples were injected with an Eppendorf pipette with a 200-μL tip into 96-well plates (150 μL/well). Each volunteer sample was exposed to all electroporation protocols in triplicate. The IRE protocol was as follows: 1 burst consisted of 216 bipolar pulses lasting 2 μs, with 5-μs pauses between the pulses (Figure 1D and 1E). Each burst was repeated 20 times with a 1-second pause in between. Electric fields ranged from 250 V/cm to 1500 V/cm. All blood samples were exposed to electric fields of 250 V/cm, 500 V/cm, 750 V/cm, 1000 V/cm, 1250 V/cm, and 1500 V/cm. One blood sample served as a control (no electric field was applied to the control well). Blood samples were collected and centrifuged for plasma separation (2500*g*, 15 minutes, 2 times). Plasma was collected and frozen at −80°C. A detailed description of the blood sampling and blood processing is given in the Supplemental Methods.

The same electroporation protocol was used for the IRE of the HL-1 cell line of cardiomyocytes. Before electroporation of cardiomyocytes, the culture medium was replaced with 50 μL of fresh medium. After electroporation, 200 μL of medium was added to the cells.

Flow cytometry analysis

For red blood cell microparticle (RBCμ) analysis, we used 50 μL of purified plasma. To detect fragments of disintegrated erythrocytes, each sample was incubated with 5 μL of phycoerythrin-conjugated CD235a (glycophorin A; IgG1 clone 11E4B-7-6 KC16) and 1 μL of fluorescein isothiocyanate–conjugated modified human recombinant annexin V for 20 minutes in the dark. After incubation, 400 μL of ice-cold 1× annexin V binding buffer and 50 μL of flow-count fluorospheres were added to each specimen and mixed gently. All reagents were purchased from Beckman Coulter (Brea, CA). Further details are presented in the Supplemental Methods and Supplemental Figure S4.

Analysis of cell-free hemoglobin

Because of the very high hemolytic range, we used the Harboe direct spectrophotometric method.[13] This method is based on the absorbance of oxyhemoglobin at 415 nm with a background correction for impurities by interpolating the optical densities at 380 nm (nonspecific plasma interferents)

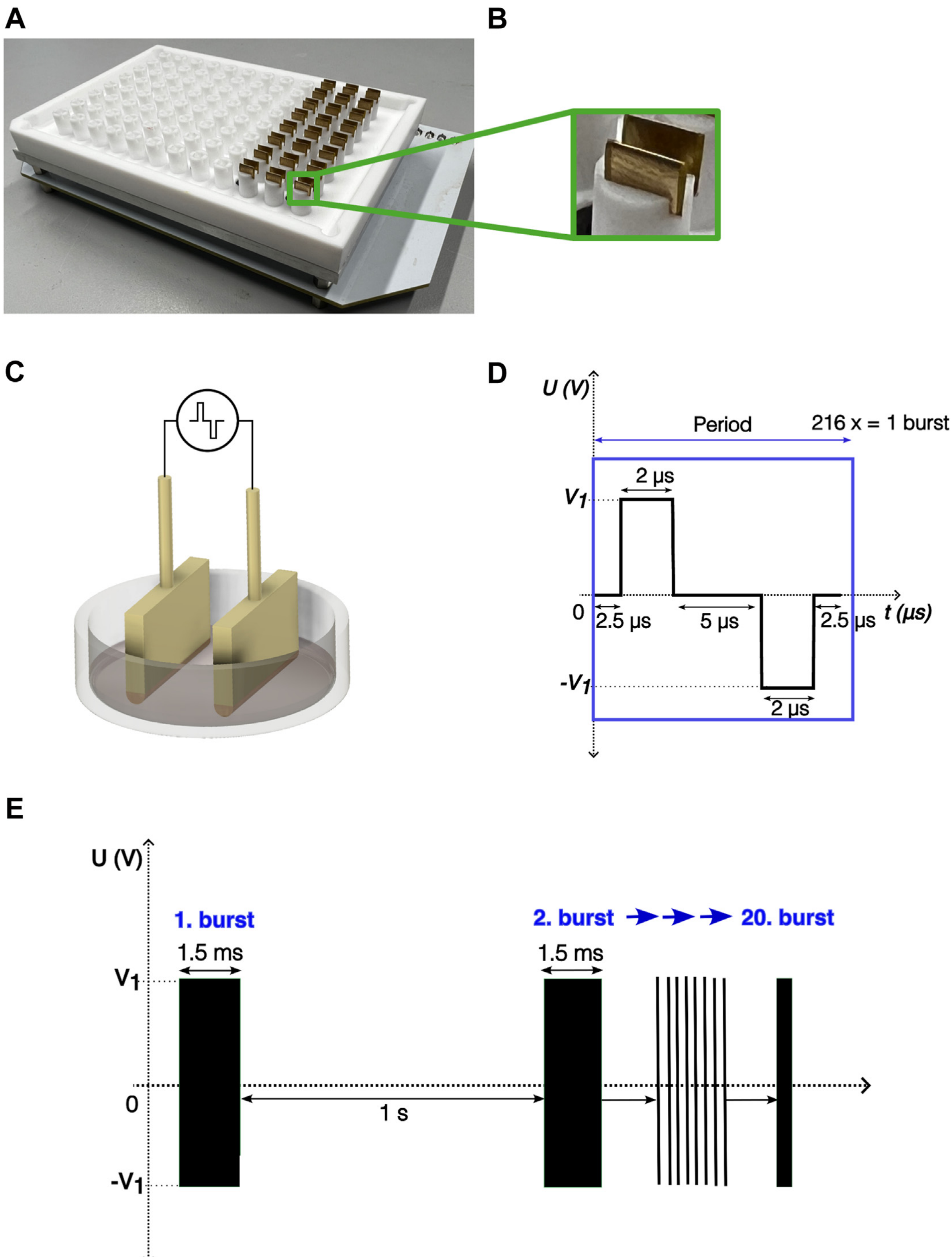

Figure 1
Electroporation setup for in vitro adherent cultures. **A:** Planar array of semiflexible noble metal electrodes with a top-layer printed circuit board and polytetrafluoroethylene (Teflon) components compatible with a 96-well cell culture plate. **B:** Detail of the electrode for 1 well. **C:** Model of the electrode system inside the 1-cell culture well. **D:** Schematic of electroporation protocol for 1 burst. **E:** Schematic of burst setup.

and 450 nm (bilirubin/albumin complexes). The plasma samples were diluted 10 times in phosphate-buffered saline. Subsequently, the absorbance was measured as mentioned before. The concentration was calculated by Equation 1.

$$Hb\left(g/l\right) = \frac{k * (167.2 * A_{415} - 83.6 * A_{380} - 83.6 * A_{450})}{1000} \quad (1)$$

Cell death detection

The cells were incubated with 5 µg/mL propidium iodide for 30 minutes at 37°C (Sigma-Aldrich, St Louis, MO) and 5 µg/mL Hoechst (cat. no. 34580; Sigma-Aldrich) for 30 minutes after IRE. Cell death was studied for 1 hour after IRE with the fluorescence microscope Nikon ECLIPSE Ts2FL. A detailed description of the cell death is given in the Supplemental Methods.

Statistical analysis

All data were assessed for normal distribution by the Shapiro-Wilk parametric hypothesis test. Because the paired data of cell-free hemoglobin and RBCμ did not exhibit a normal distribution, Friedman 2-way analysis of variance by ranks with a Bonferroni correction was employed to assess the effect of field intensity. The Wilcoxon signed rank test was used to evaluate differences between the negative control (no IRE) and each IRE-exposed sample. Because the data for cell death had a normal distribution, the 1-way analysis of variance was employed to evaluate the influence of electric field strength on cell death. Accordingly, a paired *t*-test was used to compare the negative control and each electric field intensity. The level of statistical significance for all analyses was $P = .05$. In the Results section, the data without normal distribution are described by the median with interquartile range. The data with normal distribution are described by the mean with SD. The data in the figures are presented as the spots graph with the median marked. All statistical analyses were carried out with MATLAB R2023a (MathWorks, Natick, MA).

Results

Exposure to higher intensity electric fields increases cell-free hemoglobin

The in vitro exposure of blood samples to stepwise increasing IRE induced a significant release of cell-free hemoglobin ($P < .0001$; Figure 2). A representative image of plasma after electroporation is depicted in Figure 2A. Compared with controls, the concentration of cell-free hemoglobin did not increase after exposure to a lower intensity electric field (0–750 V/cm); however, starting with 1000 V/cm, a significant increase in cell-free hemoglobin was observed (Figure 2). Exposure to 1000 V/cm, 1250 V/cm, and 1500 V/cm fields led to increased concentrations of cell-free hemoglobin, 0.32 ± 0.16 g/L, 2.2 ± 0.96 g/L, and 5.7 ± 0.39 g/L, respectively (all $P < .01$). The observed concentrations were 4-fold, 27.5-fold, and 71.25-fold higher, respectively, than the negative control in which a concentration of 0.07 ± 0.04 g/L was seen (Figure 2). The concentration of free hemoglobin increases

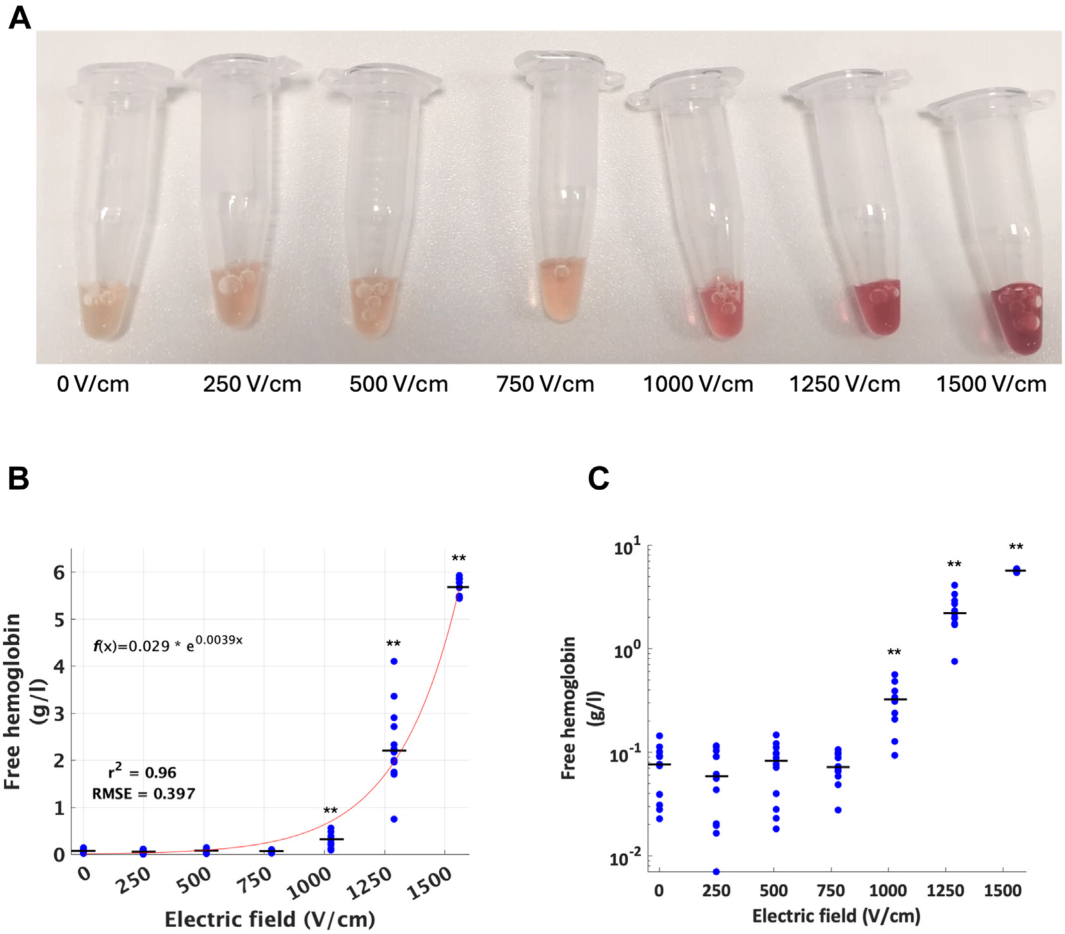

Figure 2

A: Plasma samples of blood exposed to irreversible electroporation with increased electric field. **B, C:** Concentration of cell-free hemoglobin in plasma. The data show a significant increase in free hemoglobin concentration starting with electric field strength of 1000 V/cm. Each point represents a measured value obtained from individual volunteers at the corresponding electric field strength. The *black lines* depict the median value. Free hemoglobin concentration is in linear (**B**) and logarithmic scale (**C**).**$P < .01$ based on Friedman 2-way analysis of variance with post hoc pairwise Wilcoxon signed rank test, n = 10 biologic replicates. RMSE = root mean square error.

Table 1 Median values with interquartile range of cell-free hemoglobin and RBCμ and mean value ± SD of cardiomyocyte cell death from all measured parameters for each group

Pulse voltage amplitudes (V)	Simulated electric field (V/cm)	Cell-free hemoglobin (g/L)	RBCμ (abs/μL)	Cell death (%)
0	0	0.08 ± 0.06	68 ± 79	4.4 ± 1.4
75	~250	0.06 ± 0.08	331 ± 215	4.7 ± 2.1
160	~500	0.08 ± 0.07	539 ± 462	10.2 ± 2.9
241	~750	0.07 ± 0.03	1513 ± 1569	26.5 ± 5.9
320	~1000	0.32 ± 0.16	7586 ± 14,838	44.3 ± 6.2
400	~1250	2.2 ± 0.96	52,896 ± 35,992	55.5 ± 6.9
490	~1500	5.7 ± 0.39	155,358 ± 187,556	74.5 ± 17.8

RBCμ = red blood cell microparticles.

exponentially according to the equation $f(x) = 0.029 * e^{0.0039x}$ with a correlation of 0.96 and root mean square error (RMSE) of 0.397 (Figure 2B). Figure 2C shows data with the logarithmic scale. Differences between sexes were not observed. All data are summarized in Tables 1 and 2.

Exposure of blood samples to all electric fields leads to RBCμ formation

RBCμ concentration increased at all electric field strengths during IRE ($P < .001$; Figure 3). The concentration of RBCμ was 4.9-fold, 7.9-fold, 22.3-fold, 111.6-fold, 777.9-fold, and 2284.7-fold higher in electric fields of ~250 V/cm, ~500 V/cm, ~750 V/cm, ~1000 V/cm, ~1250 V/cm, and ~1500 V/cm, respectively (all $P < .01$). Results are shown in Figure 3. Figure 3A shows all groups, and Figure 3B provides a more detailed representation of the electric field intensity up to 750 V/cm, enhancing the presentation of lower values within these groups. Figure 3C shows data with the logarithmic scale. The concentration of RBCμ increases exponentially according to the equation $f(x) = 86.2 * e^{0.005x}$ with a correlation of 0.72 and RMSE of 43179 (Figure 3B). Differences between sexes were not observed. All data are also summarized in Tables 1 and 2. A representative example of the scatter graphs is presented in Supplemental Figure S4.

IRE-induced cardiomyocyte death in vitro

The same IRE protocol with increasing electric field strength was applied on a cell line of cardiomyocytes prepared in a 96-well plate, as described previously. Whereas electric fields ~250 V/cm and ~500 V/cm did not induce cell death, a statistically significant induction of cell death was present at ~750 V/cm. The proportion of dead cardiomyocytes increased with applied voltages as follows: 27% ± 6%, 44% ± 6%, 56% ± 7%, and 75% ± 18%, corresponding to electric field intensities of ~750 V/cm, ~1000 V/cm, ~1250 V/cm, and ~1500 V/cm, respectively (Figure 4). The ratio of dead cells increases exponentially according to the equation $f(x) = 7.7 * e^{0.0015x}$ with a correlation of 0.88 and RMSE of 9.46 (Figure 4A). Figure 4B provides a view of the same data with logarithmic scale. Representative examples of fluorescence images are shown in Figure 4C. The red straight line in each picture represents dead cells due to contact with electrodes. All data are summarized in Tables 1 and 2.

Table 2 Mathematical models of individual measured parameters

Measured parameter	Equation	Correlation	RMSE
Free hemoglobin	$f(x) = 0.029*e^{0.0039}$	0.96	0.397
RBCμ	$f(x) = 86.2*e^{0.0039}$	0.72	43179
Dead cells	$f(x) = 7.7*e^{0.0039}$	0.88	9.46

RBCμ = red blood cell microparticles; RMSE = root mean square error.

Discussion

The key findings of the study can be summarized as follows:

- During in vitro PFA, statistically significant hemolysis occurred around 1000 V/cm.
- A similar electric field intensity led to considerable cell death. Destruction of a cell line of cardiomyocytes started at 750 V/cm, but the highest percentage of cell death (about 75% of exposed cardiomyocytes) occurred at 1500 V/cm.

PFA has recently gained approval from regulatory authorities for clinical use. The effect of a single high-energy electric pulse on pore formation in erythrocytes in a physiologic solution was described in the 1970s.[3] Surprisingly, although numerous preclinical animal and human studies have explored the effect of currently used PFA systems on cardiomyocytes and nonmyocardial cardiac structures, the effect on erythrocytes has not been studied. It was not until kidney failure due to hemolysis after PFA was reported that attention was drawn to the effect of PFA on erythrocytes. During catheter ablation using PF energy, the relatively broad (31 mm) catheter comes into contact not only with the endothelium of the pulmonary vein but also with the surrounding blood, leading to the release of PF energy into the surrounding blood elements. The cases of acute kidney injury due to hemolysis after PFA procedures have been reported after procedures with a large number of PF pulses.[5] Our research group from the Department of Cardiology at University Hospital Kralovske Vinohrady observed increased red blood cell (RBC) breakdown in patients undergoing catheter ablation with PFA (determined by measuring the concentration of RBCμ by flow cytometry) immediately after the procedure, followed by a decrease in haptoglobin concentration 24 hours after the procedure.[14] Furthermore, the amount of hemolysis

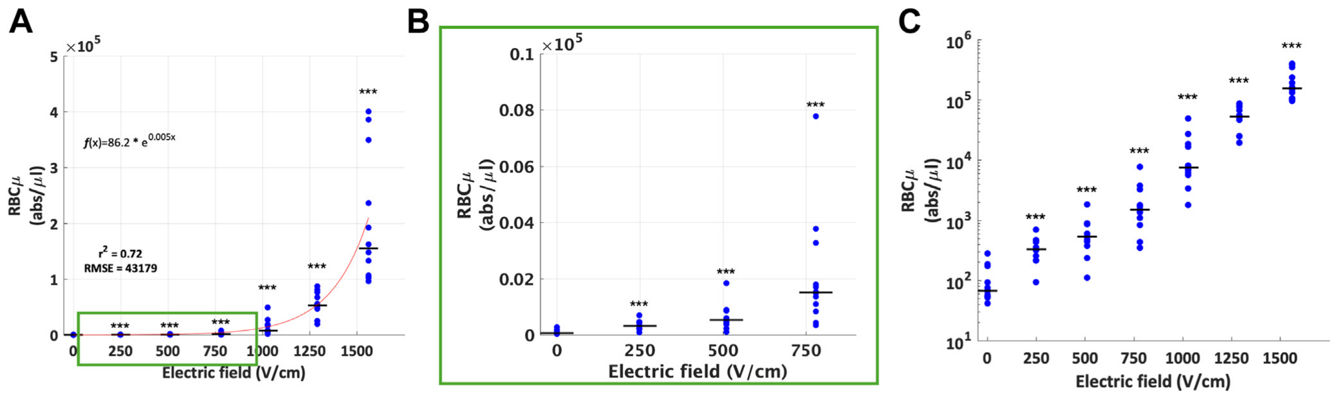

Figure 3

Concentration of red blood cell microparticles (RBCμ). The data show significantly higher RBCμ concentrations in all groups exposed to irreversible electroporation. **A:** Concentrations of RBCμ at all electric field strengths. **B:** Detail representation of lower electric field strengths up to 750 V/cm. Each point represents a measured value from individual volunteers at the corresponding electric field strength. The *black lines* depict median values. **C:** Concentrations of RBCμ at all electric field strengths, Y axis in logarithmic scale. ***$P < .001$ based on Friedman 2-way analysis of variance with post hoc pairwise Wilcoxon signed rank test, n = 12 biologic replicates. RMSE = root mean square error.

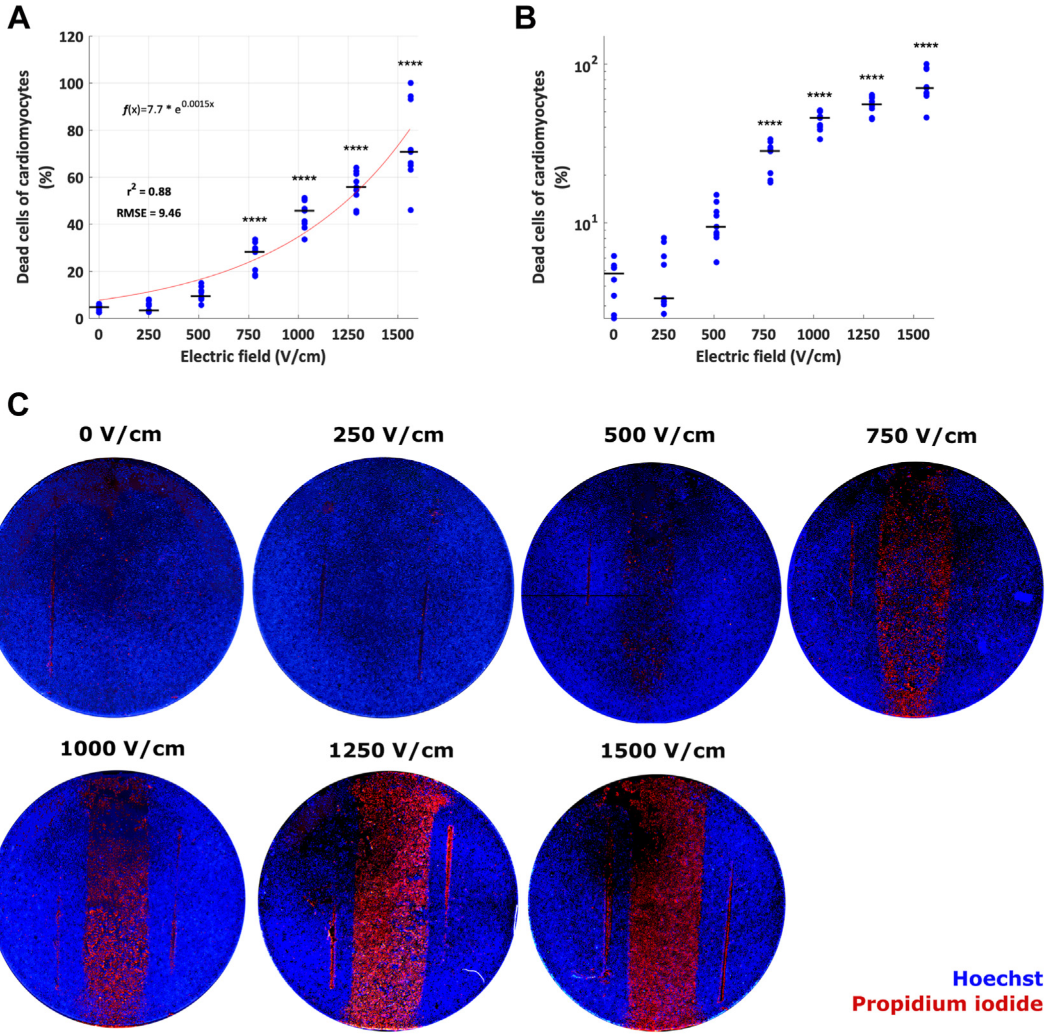

Figure 4

Cell death of atrial HL-1 mouse cardiomyocytes. The data show significantly higher cell death with an electric field strength of ~750 V/cm. Each point represents a measured value from individual volunteers at the corresponding electric field strength. **A:** Percentage of dead cells in linear and in logarithmic (**B**) scale. **C:** Representative examples of fluorescence images of dead cells. *Blue* = living cells, *red* = dead cells. The *black lines* depict the median values. ****$P < .01$ based on a 1-way analysis of variance with post hoc Dunnett test, n = 9 biologic replicates. RMSE = root mean square error.

was related to the number of PF applications. Similarly, Nies et al[15] described an increase in cell-free hemoglobin that was dependent on the number of PF applications, documenting the dose-dependent effect of PF on hemolysis.

Studying the effects of IRE in vitro let us assess and compare electric field strength thresholds that injure erythrocytes and cardiomyocytes. Moreover, in the future, the possibility of modifying the characteristics of the PF pulse in our PF generator to match those commonly used in clinical practice (as reported by the manufacturer) will enable us to test different electric field strengths and pulse durations on cardiomyocyte and erythrocyte death.

In vitro effect of PF energy on erythrocytes

An electrode system compatible with 96-well culture microplates was used to expose blood samples and a cell line of cardiomyocytes to an electric field. The electrode system created a highly homogeneous distribution of the electric field; 50% of the area between the electrodes in the culture well produces a target electric field of ±4% (Supplemental Figure S3C and S3D). Blood samples were exposed to short (2-μs) bipolar pulses structured as a set of microsecond-scale pulses of 20 bursts, each consisting of 216 pulses at a frequency of 1 Hz. The protocol follows the general principle of PFA, that is, an application of a fast sequence of high-intensity, short-duration bipolar electrical impulses.[12] Our protocol does not correspond to any specific PFA protocol currently approved for clinical practice. However, because it operates on a general principle of PFA (ie, a sequence of high-intensity electrical pulses of short duration), the results for cardiomyocyte death and hemolysis in our experiment are more comparable to in vivo conditions than results of experiments using a single electric pulse on erythrocytes in a physiologic solution.[3] The electrical conductivity of isolated erythrocytes in a physiologic solution and that in whole blood differ significantly, and electrical conductivity is an essential parameter that affects the outcome of PFA. Moreover, studies from the 1970s examined the effect of only a single, isolated electrical pulse, which does not correspond to the currently used in vivo systems that employ a sequence of multiple high-energy pulses.

Owing to the different electrical conductivity of blood cell culture media and cardiomyocytes, slightly different electrical fields were used in the erythrocytes and cardiomyocytes in our experiments. Electric field strengths of 248 V/cm, 510 V/cm, 780 V/cm, 1027 V/cm, 1289 V/cm, and 1560 V/cm were used for blood samples, and strengths of 250 V/cm, 513 V/cm, 783 V/cm, 1031 V/cm, 1291 V/cm, and 1564 V/cm were used in the experiments with cardiomyocytes (for details, see the Supplemental Methods). Because of the minimal differences in electric field strength between blood and cardiomyocytes, these variations did not significantly affect either the rate of cell death or the rate of hemolysis.

A significant rate of hemolysis seen as increased concentrations of cell-free hemoglobin was observed starting with electric field strengths of 1000 V/cm; increased concentrations of RBCμ were observed with an electrical field of 250 V/cm. For example, an electric field of 1500 V/cm resulted in a concentration of cell-free hemoglobin of 5.7 g/L. With regard to RBCμ, which are fragments of damaged RBCs, concentrations were significantly increased with all applied electric field intensities, with the increase becoming more pronounced starting at an IRE of 750 V/cm. Under clinical conditions, high amounts of cell-free hemoglobin caused by rapid hemolysis can lead to tubular injury and even acute kidney injury.[16,17] Our in vitro study cannot show the safe threshold for in vivo application of PF energy. However, our data clearly indicate that increasing the electric field intensity by short bipolar pulses contributes to significantly increased erythrocyte destruction. In addition, our data suggest that monitoring RBCμ by flow cytometry can detect IRE-associated RBC breakdown earlier than by measuring free hemoglobin in plasma. Thus, it may represent a more sensitive parameter of RBC damage than free hemoglobin.

A study from the 1970s demonstrated that applying a single 20-μs pulse generating a transient electric field of 2000 V/cm or higher can induce rupture of isolated erythrocytes. The investigators attributed this effect to an increase in intracellular ion concentration leading to elevated osmotic pressure.[3] Another study observed local elongation of attached isolated erythrocytes on a glass coverslip induced by high-frequency (MHz) electric pulses, where the length of 1 pulse is approximately 1 μs with an electric field strength of 2000 V/cm without RBC rupture.[18] RBC rupture was observed only when the electric field was increased to 5000 V/cm. Another study observed the induction of hemolysis in isolated mouse RBCs after exposure to a single 300-μs pulse and an electric field strength of about 1100 V/cm.[19] These studies confirmed that the length of the electrical pulse and the strength of the electric field can cause RBC rupture. Our findings corroborate the potential hemolytic effect of PFA under in vitro conditions and expand the threshold for erythrocyte and cardiomyocyte death by using not a single electrical impulse but a sequence of impulses similar to those used during PFA.[12]

Cardiomyocyte and in vitro PF energy

When cardiomyocytes were exposed to the same electric field strengths, a statistically significant induction of cell death started at 783 V/cm, which caused death in 27% of all cardiomyocytes. Although this proportion of cell death was statistically significant in our in vitro conditions, ablation of only 27% of cardiomyocytes is unlikely to be clinically significant during catheter ablation. Effective ablation lesions should be close to 100% of targeted cardiomyocytes. The rate of cell death increased with increasing electric field strength, with cell death present in 75% of cardiomyocytes at 1500 V/cm. Although it is challenging to translate these in vitro results to clinical settings, 75% cardiomyocyte death is certainly closer to a clinically meaningful effect. Cardiomyocyte death after high-voltage pulses has been studied by others. Casciola and coworkers[20] demonstrated an increasing area of dead induced pluripotent stem cell–derived cardiomyocytes with increasing electric field strength and number of electric pulses. The study observed cell death at 820 V/cm using 300 pulses of 5 μs duration and 10 Hz frequency.[20] A study by Baena-Montes and coworkers[7] observed effective induction of cardiomyocyte cell death at an electric field intensity of 750 V/cm (similar to our study) and a pulse length of 100 μs. These results suggest that the electric field intensity required to destroy cardiomyocytes effectively is also sufficient to induce hemolysis. However, the mechanism of cardiomyocyte cell death may involve slightly different mechanisms, and further research is needed to show whether all IRE protocols associated with effective cardiomyocyte death are also associated with hemolysis. Unlike erythrocytes, cardiomyocytes form a syncytium in tissue culture, with no cells in suspension. The mechanism of cell death is likely due to the formation of pores in the cardiomyocyte cell membrane and subsequent triggering of cell death mechanisms. Further studies and a detailed examination of different electroporation protocols are needed to investigate ways to effectively induce cardiomyocyte cell death while minimizing hemolysis. These protocols will vary not only in electric field intensity but also in the length, number, and shape of pulses.

Study limitations

The effect of PF energy on RBCs and cardiomyocytes is influenced by the pulse length, number of pulses, pause duration between pulses, and intensity of the electric field. Because the characteristics of the pulse in our in vitro PF generator do not correspond to any particular approved device, our data cannot be easily extrapolated to any currently used or developed system. In addition, in vitro blood flow is absent, unlike in catheter ablation procedures in human patients. The design of the electrodes is different from those used in the clinical setting. The in vitro induction of cardiomyocyte cell death involved only a thin layer of cells, precluding the analysis of cardiomyocyte cell death in a 3-dimensional model.

Conclusion

In our study, electric field strength energy around 1500 V/cm is required to effectively induce cardiomyocyte cell death. However, this setting also leads to significant erythrocyte breakdown, which starts with electric field strength of around 1000 V/cm.

Clinical implications

Our proposed approach enables comprehensive testing of a diverse array of electroporation protocols to investigate the development of RBC hemolysis in vitro. The data demonstrate that hemolysis can be induced on the basis of the electric field strength value with use of short bipolar pulses, which are common in clinical practice. Therefore, it is crucial to assess a specific electroporation protocol in vitro for its potential hemolytic effects, thereby enhancing the safety of the procedure.

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Appendix

Supplementary data

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.hrthm.2024.08.019.

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