Human *in vitro* model for preclinical evaluation of pulsed electric field-based non-thermal devices for cardiac ablation

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Abstract

Atrial fibrillation (AF) can lead to severe complications. Pulsed electric field (PEF) applied to biological tissues can increase cell membrane permeability and cause non-thermal irreversible cell damages, a phenomenon known as irreversible electroporation (IRE). Relative to the thermal ablation techniques, IRE has the potential to result in reduced treatment times while eliminating off-target tissue effects, thus increasing patient safety. Here, we present a novel approach to quantify the electroporation thresholds and lesion extension for a wide range of PEF parameters on cardiomyocytes and on cells from organs adjacent to the heart, under controlled experimental conditions.

Introduction

Atrial Fibrillation (AF) originates in the upper chambers of the heart (atria) and results in rapid, irregular heartbeat (90 - 170 beats per minute). AF can lead to blood clots, stroke, heart failure and other heart-related complications. AF is the most common cardiac arrhythmia with at least 2.7 million Americans affected. A population study from 2007 estimates that the number of Americans afflicted by AF will increase to more than 10 million by 2050. Cardiac ablation is a procedure used to destroy the faulty electrical pathways responsible for AF in patients that are not responsive to pharmaceutical treatments. Currently, termination of a faulty electrical pathway requires energy in the form of either heat (like radiofrequency - RF- ablation) or extreme cold (cryogenic, like cryoballoon ablation) delivered through a catheter inserted through a vein or artery in the groin. While these procedures are relatively safe and effective, the long-term success rate of RF ablation is between 40-60% after a single procedure, and about 70% after multiple procedures. Thermal cardiac ablation is also associated with rare, but serious complications with potentially fatal consequences related to off-target damage to surrounding tissues. Unintended injury to nontargeted tissues is related to the lack of control over the extent of the lesion formation.

Pulsed Electric Fields (PEFs) have been extensively used to permeabilize cell membranes, a phenomenon known as electroporation. The increase of cell permeability following PEF application can be either reversible or irreversible, depending on the electric Field Threshold (EFT) reached in the region to be treated. Irreversible electroporation (IRE) has been widely exploited as an ablative modality for the treatment of tumors due to its unique ability to induce non-thermal cell death, even in close proximity to vasculature and nerves. Compared to traditional thermal ablation techniques, IRE has the potential to reduce treatment times while eliminating off-target tissue effects, creating consistent and predictable irreversible lesions. IRE has further been known to cause cardiac lesions without damage to the extracellular matrix in ex vivo tissue scaffolding. This ability to increase patient safety has resulted in a surge in the production of new medical devices that use IRE for cardiac ablation. While these same studies have highlighted the inherent safety and effectiveness of IRE cardiac ablation, a comprehensive assessment of the effects of varying PEF parameters on cardiac and surrounding tissues is missing. Currently, manufacturers develop customized, preclinical bench testing methods to provide initial safety and performance reports, however the variability in testing environments and differences in testing methodologies impacts the ability to optimize the treatment as well as to review these results from a regulatory perspective. Standardized tools for the preclinical evaluation of novel IRE cardiac ablation devices would prompt treatment optimization, facilitating the regulatory review process and the access of life-preserving therapies to patients in need.

Objective

This work aims to develop a standardized laboratory method to evaluate the safety and effectiveness of IRE ablation devices using human induced pluripotent stem cell cardiomyocytes (hiPSC-CMs) as a cardiac model.

Materials and Methods

hiPSC-CMs (iCell Cardiomyocytes2, Fujifilm Cellular Dynamic, Inc) and HESM (human esophageal smooth muscle, ScienCell) cells were evaluated in a monolayer format using NanoFiber plates. Pulses were delivered by an FID pulse generator (FID GmbH, Germany) through contact electrodes (2-mm interelectrode distance). The pulse parameters evaluated in this study include: waveform - rectangular unipolar and bipolar; duration - 0.5 to 500 µs; number - 1 to 300; frequency - 0.01 to 1 kHz; train number - 1 to 10. Reversible electroporation was quantified by florescence imaging with YO-PRO-1 (YP1) added before exposure, while irreversible electroporation with propidium iodide (Pi) added 10 minutes prior imaging. Images were acquired 4 h after treatment. Electric field (E) distribution maps were generated with numerical simulations, Sim4Life v3.2 (Zurich Med Tech, Swiss).

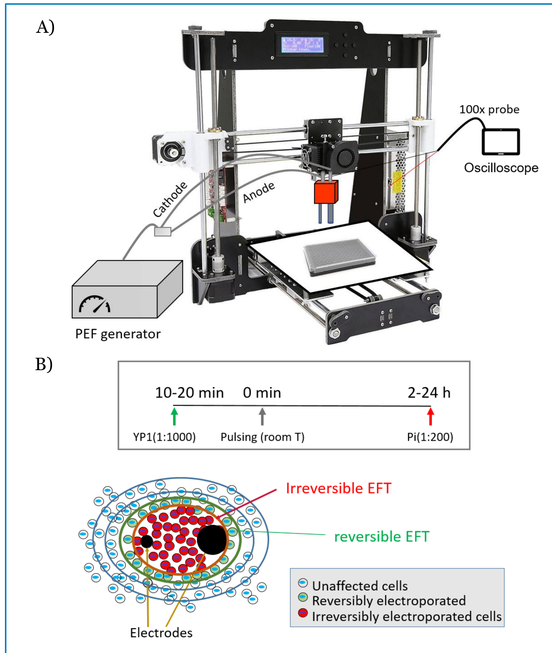

Figure 1. A) Schematic of the high throughput experimental setup for PEF delivery to cell monolayers. The main components of the setup are a PEF generator, PEF delivery electrodes, a robotic arm, an oscilloscope. B) Experimental timeline and diagram of the electroporation threshold determination obtained by overlaying the E map with a fluorescence image of a pulse-treated cell monolayer.

Results and Discussion

We tested the feasibility of the proposed approach on hiPSC-CM using as endpoint the cell death by PI uptake at 4h after exposure. Data was collected from n = 3 independent experiments on cell monolayers cultured in 96 well plates.

hiPSC-CM were exposed to a train of rectangular unipolar pulses of 3 and 5 µs duration. The number of pulses was either 60 or 120 delivered at 10, 100 and 100 Hz repetition frequency. While the voltage applied (V) to the electrodes was 100, 150, 200, 250 V. Sham exposure (0 V) enabled the determination of the extension of cell (mechanical) damage due to the imprint of the electrodes on the cell monolayer.

For a train of 120 unipolar pulses of 3 µs duration delivered at 1 kHz repetition frequency, the E field threshold for IRE was 0.9 kV/cm. The EFT was determined by overlapping the E field distribution numerically computed (Figure 2) to the fluoresce image (Figure 3A). Since the E field was calculated for 1 V of difference between the electrodes, the isoline corresponding to the irreversible effect was scaled by the V applied during the experiments to obtain the EFT.

For the same train of pulses, the ablation lesion increased from 0.33 ± 0.03 to 3.70 ± 0.24 mm² when the V applied ranged from 100 to 250 V (Figure 3B). Reducing the number of pulses by 50% decreased the ablation area by 30% (data not shown). While, increasing the duration to 5 µs resulted in a 1.4-fold increase of the lesion (data not shown). A negligible effect was observed when the repetition frequency was decreased from 1 kHz to 10 and 100 Hz (data not shown).

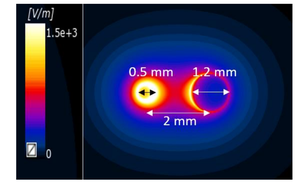

Figure 2. Electric (E) field distribution generated by the electrodes in the plane of the cell monolayer. To define the EFT the distributions values obtained by modeling (1 V) was scaled by the experimental value of V.

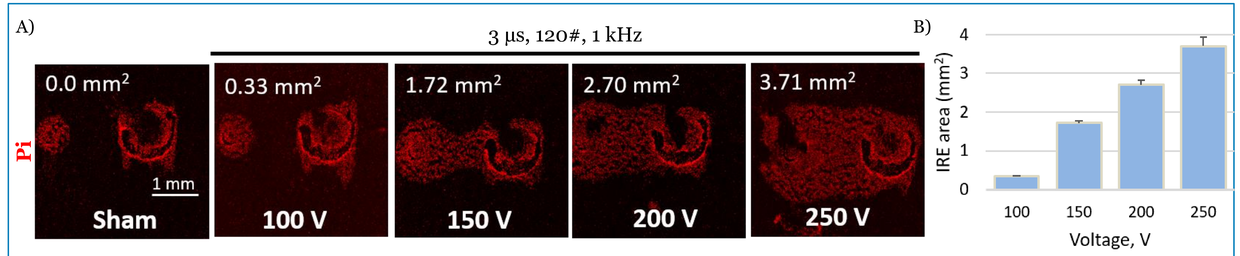

Figure 3. A) Fluorescence images of PEF-treated hiPSC-CMs. B) For N = 3 independent experiments, the surface of lesion increased with the V applied.

When HESM cells were exposed to the same set of pulse parameters than hiPSC-CM, ~4folds higher V applied was necessary to produce a similar IRE lesion (Figure 4, Pi indicator).

Under the PEF conditions studied, while we observed reversible electroporation through YP1 uptake by HESM cells, hiPSC-CM did not show signs of YP1 uptake beyond the IRE lesion (Figure 4). Further investigation is needed to confirm these results.

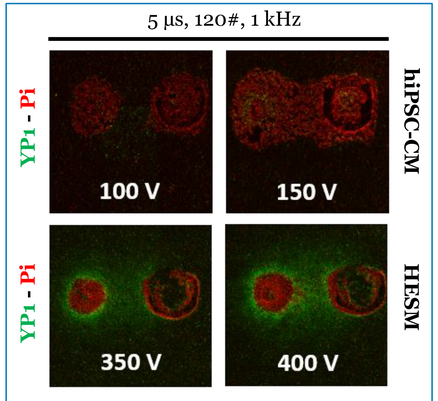

Figure 4. hiPSC-CM showed a lower irreversible EFT than HESM. Cells were exposed to a train of 5 µs long, 120# unipolar pulses @ 1 kHz. 100 and 150 V were applied to hiPSC-CM (upper panel), while 350 and 400 V to HESM (lower panel).

Conclusion

These results demonstrate that our approach is capable of characterizing the effects of IRE on hiPSC-CM. The data collected suggest that there is an increase of the IRE lesion as well as of the EFT with increasing voltage, pulse duration, pulse number, while the repetition frequency seems to have less impact. Our results also suggest that to create the same lesion in HESM compared to hiPSC-CM exposed to the same PEF treatment, a ~4 times higher V applied is needed.

This work will be extended to evaluate the effects of a broad range of PEF parameter on different human cell lines anatomically adjacent to the heart to address safety and efficacy of IRE cardiac ablation. The proposed study will help FDA to be on the leading edge of this new technology and to simplify the regulatory process.

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