Quantifying Electrical and Optical Signatures of Global Ischemia in Whole Human Hearts for Ex Situ Viability Assessment

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Abstract

Heart transplantation remains the definitive therapy for end-stage heart failure, yet one in five patients die while waiting. Expanding the donor pool via donation after circulatory death (DCD) is limited by the lack of quantitative physiological criteria to assess post-ischemic myocardial viability. Current DCD protocols rely on empirical cutoffs for functional warm ischemia (e.g., systolic pressure and time) without direct assessment.

We developed an ex-situ human heart platform integrating dual-camera optical mapping with multi-electrode torso-tank electrocardiography (ECG) to simultaneously quantify the heart's electrical response to ischemia and reperfusion. In a procured human donor heart, both global no-flow ischemia and functional ischemia (perfusion pressure < 30 mmHg) result in significant shortening of the action potential duration (APD) and increased dispersion of spatial repolarization. These optical findings were accompanied by progressive QRS widening and loss of isoelectric ST-segment on tank ECG. Upon reperfusion, APD recovered exponentially, paralleling the normalization of ECG morphology.

These results introduce a quantitative metric of electrical recovery, providing a basis for real-time ex-situ viability assessment of DCD donor hearts.

1. Introduction

Despite advances in perfusion technology, there remains no quantitative method to determine when myocardial injury in DCD donors becomes irreversible. Current procurement protocols rely on empirical clinical surrogates, such as systolic blood pressure thresholds and the duration of the agonal phase. At the same time, intraoperative evaluation depends on assessment of akinetic regions or lactate levels as indirect markers of ischemic metabolism. These methods are severely limited as they fail to capture the spatiotemporal evolution of ischemia, and no real-time electrophysiological assessment is currently available during the in-situ functional ischemic phase preceding procurement. Such a quantitative assessment could define the onset, progression, and reversibility of ischemia, for accurately predicting post-procurement recovery.

Ischemia rapidly depletes ATP, activating K_{ATP} channels and increasing K^+ efflux, which shortens the action-potential plateau and reduces Ca^{2+} influx, thereby decreasing contractility. While initially adaptive, prolonged ischemia leads to increased diastolic stiffness and lowered perfusion pressure, further restricting coronary flow. Because these electrical changes precede mechanical failure, they offer a sensitive window for detecting the onset and reversibility of injury. Optical mapping of transmembrane voltage using voltage-sensitive dyes provides high-resolution visualization of APD, repolarization dynamics, and spatial dispersion. Simultaneously, multi-electrode ECG recordings capture the corresponding extracellular potentials that translate to clinically measurable surface potentials. By directly linking cellular action potentials with extracellular ECG features, this approach provides a mechanistic bridge to clinically quantifiable signals.

Here, we developed an ex-situ human heart platform that integrates dual-camera optical mapping with multi-electrode torso-tank ECG to quantify the electrophysiological effects of ischemia and recovery. Using a procured human donor heart from a DBD donor, we simulated conditions relevant to DCD procurement by examining both global zero-flow ischemia and functional ischemia (mean pressure < 30 mmHg). We hypothesized that quantifiable changes in APD shortening and recovery kinetics would provide robust electrophysiological markers of reversible ischemia, and that corresponding ECG features, particularly QRS widening and ST-segment deviation, would mirror APD changes at the whole heart level. This optical–electrical framework establishes the foundation for developing noninvasive, physiology-based metrics to assess electrical viability of donor hearts in real time during DCD procurement.

2. Methods

2.1. Whole-heart platform

Experiments were performed using a healthy DBD donor heart deemed unsuitable for transplantation,

procured through LifeNet Health under an approved agreement. Immediately after excision, the heart was arrested with cold cardioplegia (4 °C) and transported to the laboratory. Upon arrival, it was cannulated via the left and right coronary ostia and perfused with a modified Krebs–Henseleit bicarbonate pH buffer equilibrated with 95% O_2 / 5% CO_2, at constant pressure (70 mmHg).

The heart was mounted in a transparent hexagonal tank filled with a circulating, temperature-controlled (37 °C) Krebs–Henseleit solution. Mechanical contractions were inhibited with 3 µM (−)-Blebbistatin. This setup enabled simultaneous optical and electrical recordings using dual high-speed cameras and 15-channel electrode arrays (Figure 1), providing high-resolution visualization of activation and repolarization dynamics during baseline, ischemia, and reperfusion [1].

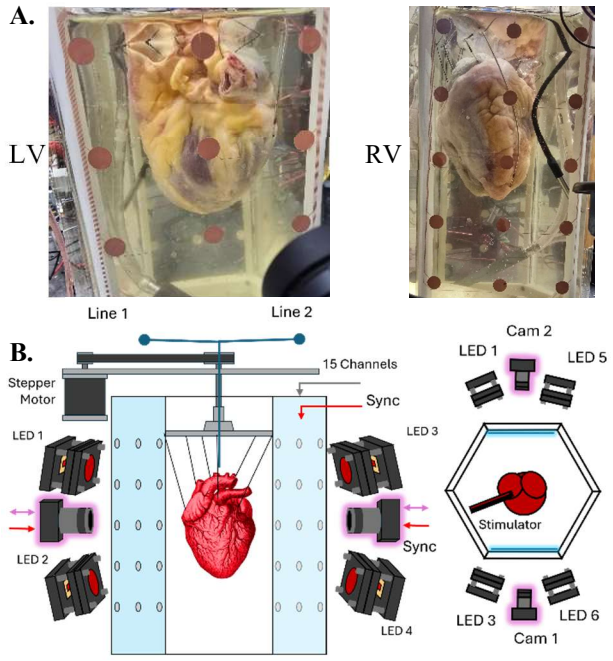

Figure 1. A. Photographs of the human donor heart suspended in the hexagonal tank, showing the left (LV) and right (RV) ventricular surfaces through the transparent 15-channel electrode array faces. B. Illustration of the dual-camera imaging system, featuring 650 nm LED illumination, synchronized acquisition (Sync) of optical (transmembrane voltage) and electrical (torso-tank ECG) signals, and 15-channel electrode arrays positioned for high-resolution measurement.

2.2. Optical and electrical recordings

Transmembrane voltage was recorded using the voltage-sensitive dye JPW-6003 (1.5 mg per heart). Hearts were illuminated with high-power LEDs centered at 650 nm, and the emitted fluorescence (> 700 nm) was collected by two high-speed CMOS cameras (Sony IMX278 sensors) positioned 180° apart. The cameras imaged the right and left ventricular lateral walls at raw resolution of 720×480 pixels and a frame rate of 500 Hz. Optical recordings were synchronized with electrical signals, and minor residual motion artifacts were minimized by temporal filtering during post-processing.

Torso-tank ECGs were recorded using two 15-electrode PCB arrays facing the LV and RV. Signals were acquired using an Intan RHD recording system, sampled at 4 kHz, with a passband of 0.1 Hz to 1 kHz.

2.3. Ischemia protocols

Two ischemic protocols were applied to the human DBD donor heart. For global zero-flow ischemia, coronary perfusion was stopped for 10 min while the heart was continuously paced at an 800 ms cycle length to maintain electrical activity for measurement. Perfusion was then restored for 15 min to assess recovery kinetics.

The second protocol, functional ischemia, was used to mimic the DCD agonal phase. Perfusion pressure was reduced to 30 mmHg, controlled separately for LV and RV for a total of 20 minutes. The heart was paced at an 800 ms cycle length throughout this low-pressure period, followed by a 15-minute recovery period.

2.4. Data processing and analysis

Optical signals were processed in MATLAB using custom algorithms to quantify the local APD at 80% repolarization (APD_{80}). Relative APD change was computed with respect to baseline prior to ischemia. Recovery following reperfusion was quantified by fitting a mono-exponential model to the APD_{80} time series to determine the recovery time constant (τ) for global ischemia. For electrical analysis, signals from all 30 electrodes were averaged and referenced to a Wilson Central Terminal, formed from the composite mean of all tank electrodes. ECG waveforms were used to assess morphology changes in the QRS complex, ST segment, and T-wave during ischemia and reperfusion.

3. Results

3.1. Global zero-flow ischemia

During global no-flow ischemia (10 min), spatially variable yet globally synchronized APD_{80} shortening was observed across the LV and RV surfaces, followed by complete recovery upon reperfusion (Figure 2). Dual-camera imaging captured concurrent changes across LV and RV, demonstrating that ischemic shortening and recovery kinetics were spatially concordant across LV

and RV regions. Time-resolved APD_{80} heatmaps revealed a gradual decline during ischemia and exponential recovery within 15 min of reperfusion. The APD_{80} shortening was significant, reaching ~30% (~3% per minute for LV) and ~35% (RV), with complete restoration of baseline activation patterns after reperfusion.

Global no-flow ischemia exhibited a near-linear early decline in APD_{80}, indicating a constant rate of electrical adaptation rather than abrupt failure. This linearity implies preserved viability, as the myocardium continued to adjust uniformly to reduced oxygen availability. A mono-exponential fit over the observed window yielded a time constant of $\tau \approx 25$ min of early electrical deterioration. The relatively long τ reflects reduced oxygen consumption in the mechanically arrested heart, where metabolic demand was minimized. In a contracting heart, this timescale would be substantially shorter. Upon reperfusion, APD_{80} recovered more rapidly ($\tau \approx 8.5$ min), implying preserved metabolic reserve and complete reversibility of early ischemic changes.

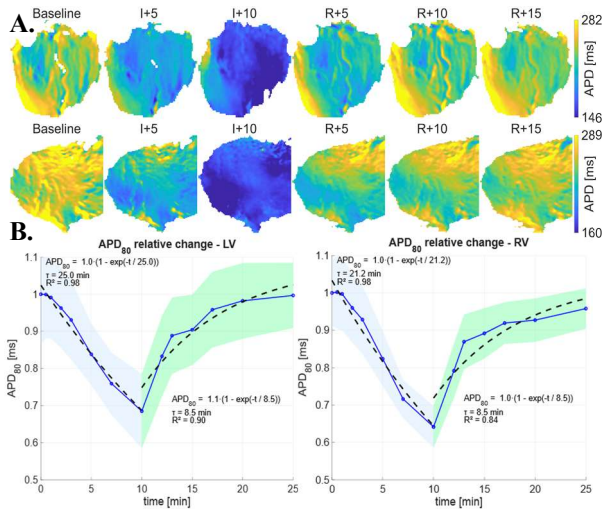

Figure 2. A. APD_{80} maps from LV and RV surfaces show progressive APD shortening during 10 min of global no-flow ischemia and with full recovery after 15 min of reperfusion. The color scale spans the 3rd to 97th percentile of APD values. B. APD_{80} kinetics reveal symmetric recovery across both ventricles with a time constant of ~8.5 min. Shaded areas denote the mean ±1 standard deviation. Dashed lines show exponential fits.

Simultaneous pseudo-ECG recordings from electrodes facing the LV lateral surface in the torso-tank model revealed waveform changes consistent with optical mapping observations of APD shortening during ischemia (Figure 3). At baseline, all leads displayed narrow QRS complexes, an isoelectric ST-segment and upright, smooth T-waves. This pattern represents the electrical manifestation of healthy ventricular function, characterized by rapid depolarization and a synchronous repolarization sequence. The flat, well-defined ST segment corresponds to the sustained plateau phase of the action potential, during which the ventricular myocardium remains uniformly depolarized.

After 10 minutes of global ischemia, the pseudo-ECG exhibited marked alterations. The QRS complex was prolonged, reflecting slowed ventricular conduction due to the partial depolarization and inactivation of fast sodium channels in ischemic tissue. Consistent with optical mapping, the loss of the action potential plateau and subsequent APD shortening caused heterogeneous repolarization, with ischemic regions repolarizing prematurely while surrounding tissue remained depolarized. This nonuniform recovery of excitability eliminated the distinct isoelectric ST segment, merging it with the broadened QRS–T complex. The resulting flattened or indistinct T-wave directly reflected the enhanced APD dispersion and highly heterogeneous repolarization wavefronts, which fail to generate the coherent electrical vector necessary for a normal T-wave morphology. Upon reperfusion, QRS, ST-segment, and T-wave morphology returned toward baseline, confirming the complete reversibility of the electrical changes.

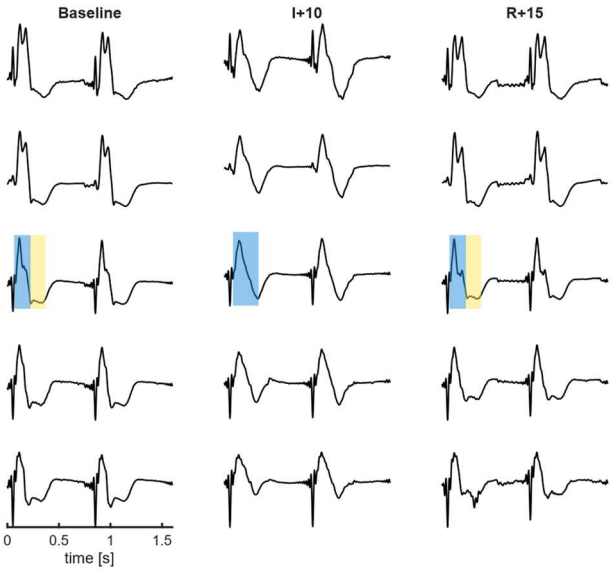

Figure 3. Representative pseudo-ECGs (each trace horizontally averaged from a 5×3 matrix) under baseline, 10 min ischemia (I+10), and 15 min reperfusion (R+15). At baseline, the QRS complex (blue) is narrow, with distinct isoelectric ST segment (yellow) and T-wave. During ischemia, QRS widens, and the ST segment merges with the broadened QRS-T. Morphology recovers after reperfusion.

3.2. Functional ischemia

To model the ischemia during the agonal phase of DCD donation, coronary perfusion pressure was reduced to 30 mmHg, for 20 minutes. As in the zero-flow ischemia the flow reduction was characterized by a spatially heterogeneous APD shortening across the LV (Figure 4). Quantitative analysis of the APD_{80} time course revealed a mean APD_{80} shortening of ~20% (1% per minute) from baseline during functional ischemia (FI+20), with full recovery upon pressure restoration to 70 mmHg.

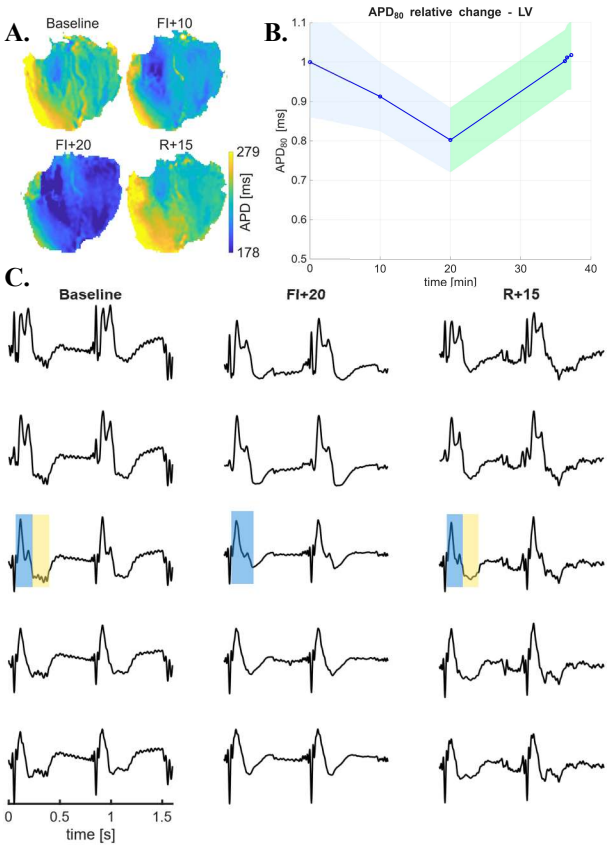

Figure 4. A. APD maps of LV. B. Mean APD line plots showing APD_{80} shortening during functional ischemia and recovery. C. Pseudo-ECG tracings confirming QRS widening and ST segment elimination at FI+20, followed by complete recovery upon pressure restoration.

Pseudo-ECG recordings mirrored the electrophysiological changes observed during zero-flow ischemia. At baseline, QRS complexes were narrow, followed by a distinct, flat ST segment and upright T-waves. During functional ischemia (FI+20), the QRS complexes broadened, and the ST segment became indistinct, merging with early repolarization. After reperfusion (R+15), QRS width, ST level, and T-wave morphology progressively returned toward baseline, confirming the transient nature of functional ischemia.

3.3. Discussion and conclusions

This study demonstrates the feasibility of simultaneous optical and electrical mapping to quantify electrophysiological signatures of ischemia and recovery in perfused human hearts. The findings reveal distinct kinetics: APD shortened by ~3% per minute during no-flow ischemia, about three times faster than during functional ischemia. Although electrical recovery was complete after 10 minutes of warm no-flow ischemia in the mechanically uncoupled heart, such a duration is clinically unacceptable for DBD procurement. This underscores the limitation of time-based criteria (e.g., the 30-minute threshold for DCD acceptance), which overlook real-time measures of viability. Together, these optical–electrical data establish a quantitative framework for real-time viability assessment, bridging mechanistic insights with clinically measurable ECG features.

4. Limitations

While this study demonstrates feasibility, it is subject to several limitations. The heart was mechanically uncoupled and perfused at constant pressure to stabilize optical recordings, which precluded assessment of contractile recovery. While this design isolates electrophysiological effects, it omits mechanical feedback and neurohumoral influences present in vivo. Experiments were performed ex-situ in a torso-tank geometry that differs from in-situ electrical conditions.

Acknowledgments

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References

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