Transmural Dispersion of Repolarization in Failing and Nonfailing Human Ventricle

Alexey V. Glukhov, Vadim V. Fedorov, Qing Lou, Vinod K. Ravikumar, Paul W. Kalish, Richard B. Schuessler, Nader Moazami, Igor R. Efimov

Rationale: Transmural dispersion of repolarization has been shown to play a role in the genesis of ventricular tachycardia and fibrillation in different animal models of heart failure (HF). Heterogeneous changes of repolarization within the midmyocardial population of ventricular cells have been considered an important contributor to the HF phenotype. However, there is limited electrophysiological data from the human heart.

Objective: To study electrophysiological remodeling of transmural repolarization in the failing and nonfailing human hearts.

Methods and Results: We optically mapped the action potential duration (APD) in the coronary-perfused scar-free posterior-lateral left ventricular free wall wedge preparations from failing (n = 5) and nonfailing (n = 5) human hearts. During slow pacing (S1S1 = 2000 ms), in the nonfailing hearts we observed significant transmural APD gradient: subepicardial, midmyocardial, and subendocardial APD80 were 383±21, 455±20, and 494±22 ms, respectively. In 60% of nonfailing hearts (3 of 5), we found midmyocardial islands of cells that presented a distinctly long APD (537±40 ms) and a steep local APD gradient (27±7 ms/mm) compared with the neighboring myocardium. HF resulted in prolongation of APD80: 477±22 ms, 495±29 ms, and 506±35 ms for the subepi-, mid-, and subendocardium, respectively, while reducing transmural APD80 difference from 111±13 to 29±6 ms (P<0.005) and presence of any prominent local APD gradient. In HF, immunostaining revealed a significant reduction of connexin43 expression on the subepicardium.

Conclusions: We present for the first time direct experimental evidence of a transmural APD gradient in the human heart. HF results in the heterogeneous prolongation of APD, which significantly reduces the transmural and local APD gradients. (Circ Res. 2010;106:981-991.)

Key Words: heart failure ■ repolarization ■ transmural gradient ■ optical mapping ■ connexin43

Heart failure (HF) claims more than 200,000 lives annually in the US alone.1-2 Approximately a half of these deaths are sudden and presumably caused by ventricular tachyarrhythmias. Pathophysiological remodeling of cardiac function occurs at multiple levels and includes the alterations in a host of ion channels, Ca2+-handling proteins, and proteins mediating cell-cell coupling, predisposing to arrhythmias and sudden death.3 Numerous animal models have shown the importance of such electrophysiological (EP) remodeling in the mechanisms of HF-related arrhythmogenesis.4

Prolongation of the repolarization is a hallmark of cells and tissues isolated from failing hearts independent of the cause, which has been observed in isolated myocytes5 and intact ventricular preparations.4,6 This fundamental change in myocyte biology underlies QT-interval prolongation of the surface ECG in patients with HF. The action potential (AP) prolongation is heterogeneous, resulting in exaggeration of the physiological heterogeneity of electric properties in the failing heart.1-4 These cellular EP changes were linked to downregulation of potassium currents (Ik1, IKr, IK1a, and IK1), an increase in late Na current (INaL) density, as well as significant changes in intracellular calcium handling proteins.1-3 However, despite these studies a direct mechanistic link between repolarization changes observed in isolated myocytes or on the body surface and arrhythmia genesis remained lacking, largely because of technical difficulties in assessing the functional expression of spatiotemporal repolarization changes at the intact heart level.

Transmural dispersion of repolarization has been suggested to play an important role in the genesis of polymorphic ventricular tachycardia in different animal models of HF.4,7,8 In the normal human ventricular myocardium, at least 3 different populations of cells (subepicardial, subendocardial, and midmyocardial [M cells]) are selected based on their
Taggart et al.16 have been unable to detect physiologically pronounced bradycardia as well as during early ischemia. These discrepancies were explained by the cell injury or cell-cell uncoupling caused by cell isolation or tissue dissection, by influence of local environment of cells, or by use of anesthetic agents in the in vivo studies that suppress transmural dispersion of repolarization.17–20 However, this study did not investigate the effect of HF on the transmural repolarization and presented a combination of patients with different etiology.16 In view of the EP differences between different studies and limited information from the human heart, in this study we optically mapped transmural wedge preparation from failing and nonfailing human hearts. We also mapped immunostaining for expression of connexin (Cx)43 through the LV wall.

Methods
An expanded Methods section is available in the Online Data Supplement at http://circres.ahajournals.org.

Patients Groups
Failing hearts (n=5, Online Table I) with different types of cardiomyopathy were obtained during transplantation at the Barnes-Jewish Hospital, Washington University in Saint Louis, MO. For comparison, we used nonfailing donor hearts (n=5, Online Table II), which were rejected for transplantation for various reasons, including age, early stage hypertrophy, atrial fibrillation, and coronary disease. Donor hearts were provided by the Mid-America Transplant Services (St Louis, Mo). The study was approved by the Washington University Institutional Review Board.

Explanted hearts were cardioplegically arrested and cooled to 4 to 7°C in the operating room following cross-clamping of the aorta. The arrested heart was maintained at 4 to 7°C to preserve tissue during 15 to 20 minutes delivery from the operating room to the research laboratory.

Experimental Preparation
We isolated wedges of human ventricular wall as previously described in canine heart.17,18 Briefly, after harvesting, the hearts were immediately perfused through the aorta with a cardioplegic solution (in mmol/L: NaCl 110, CaCl2 1.2, KCl 16, MgCl2 16, NaHCO3 10, 4°C). The cardioplegic perfusion washed out the blood and protected the hearts during the subsequent period of wedge isolation. We then isolated a transmural wedge from the posterior-lateral LV free wall supplied by left marginal artery (Figure 1A). The preparation was dissected several centimeters below the base of the ventricles and extended ~3 cm toward the apex (Figure 1A and 1B). The thickness of the LV was not differ between nonfailing and failing wedge preparations (16.6±1.4 mm versus 19.2±1.5 mm, respectively, P=0.26; see also Online Table III). Each wedge contained a section of coronary artery (diameter ≈1 mm) along its length, which was cannulated with the flexible plastic cannula custom made for these experiments. Major arterial leaks in the wedges were ligated with silk suture. The quality of perfusion was verified by injection of Methylene Blue dye (Sigma, St. Louis, MO). Poorly perfused tissue was trimmed from the wedges. The isolated tissues were mounted in a warm chamber with the dissected exposed transmural surface up, facing the optical apparatus. Wedges were perfused with oxygenated Tyrode solution composed of (in mmol/L): 128.2 NaCl, 4.7 KCl, 1.19 NaH2PO4, 1.05 MgCl2, 1.3 CaCl2, 20.0 NaHCO3, and 11.1 glucose, and gassed with 95% O2,5% CO2; pH=7.35±0.05. We maintained 37°C and an arterial pressure of 60 to 70 mm Hg. The preparation was fully immersed in the perfusion efflux, which assured appropriate superfusion.

Imaging System
After 20 to 30 minutes of washout, gradual warming after cold cardioplegia to 37°C, tissue recovery, and stabilization, the wedges were stained with 4 μmol/L di-4-ANEPPS (Molecular Probes, Eugene, Ore), a membrane potential-sensitive fluorescent dye having no known electrophysiological effects and used widely in optical mapping studies in hearts of many species.

We immobilized the wedges with 10 μmol/L blebbistatin (Tocris Bioscience, Ellisville, MO) which inhibits the adenosine triphosphatases (ATPases) associated with class II myosin isoforms in an actin-detached state, and thus successfully blocks cardiac contraction without any effect on electric activity, including ECG parameters.
atrial and ventricular effective refractory periods, and atrial and ventricular activation patterns in many mammalian species.\textsuperscript{21,22} We used microelectrode recordings to validate the effect of Blebbistatin in the human ventricle (see Online Data Supplement).

The wedges were paced at the endocardium by 5 to 10 ms pulses at 2× diastolic current thresholds at a pacing cycle length (CL) ranging from 4000 ms to the ventricular functional refractory period. Two Ag/AgCl electrodes were immersed into the superfusion solution, one at the epicardial and the other at the endocardial side, to document the transmural pseudo-ECG (Figure 1C).

An optical mapping system\textsuperscript{23} with a 100×100 pixels resolution MiCAM Ultima-L CMOS camera (SciMedia) collected the fluorescent light from an area of 2 to 3 cm by 2 to 3 cm (Figure 1B) on the cut-exposed transmural surface of the wedge. Optical APs were recorded from the transmural optical field of view (20×20 to 30×30 mm\textsuperscript{2}) with a spatial resolution of 200 to 300 µm/pixel at a rate of 1000 frames/sec (Figure 1C). The fluorescent signals were amplified, digitized, and visualized during experiment using specialized software (SciMedia).

Data Processing
A custom-made Matlab-based computer program was used to analyze APs offline.\textsuperscript{24} First, the signals were filtered using the low-pass Butterworth filter at 50 Hz. Activation maps were constructed from activation times, which were determined from the dV/dt\textsubscript{max} in each channel. Finally, AP duration was calculated as time difference between the activation time (dV/dt\textsubscript{max}) and 80% of repolarization (APD\textsubscript{80}).

Local APD gradient was measured as the APD difference of neighboring pixels more than the pixel length after 3×3 average filtering. To estimate size and area of islands of prolonged APD, we used criteria based on the local APD gradient with the threshold of 15 mm/ms. Maximum APD within local high-APD-gradient boundary line was identified and then used as a threshold for the island with prolonged repolarization. For detailed description of data analyses see Online Data Supplement.

Restitution Protocol
The dependence of APD\textsubscript{80} on the preceding diastolic interval (DI) was determined using the dynamic restitution protocol as described earlier.\textsuperscript{25} Preparations were paced at a constant basic CL, which was shortened from 4000 to 1000 ms in steps of 500 ms, from 1000 to 500 in step of 100 ms, and in steps of 10 ms from 400 ms until ventricular functional refractory period was reached. We observed APD alternans at short CL. To measure APD of both long and short ventricular functional refractory period was reached. We observed APD alternans at short CL. To measure APD of both long and short ventricular functional refractory period was reached. We observed APD alternans at short CL. To measure APD of both long and short

APD alternans was quantified by computing AP for 2 consecutive beats. Similarly, AP amplitude alternans was quantified by computing the amplitude differences of 2 consecutive beats as described elsewhere.\textsuperscript{26}

Histology and Immunofluorescence Labeling
Histology experiments were performed as previously described.\textsuperscript{27} Sections were stained with Masson’s trichrome (International Medical Equipment, San Marcos, CA, USA). The examples of histological staining for subepicardial, midmyocardial, and subendocardial sections are presented on Online Figure V (A). Immunolabeling was carried out as described previously.\textsuperscript{28,29,30} Sections were stained with commercially available antibodies: Rabbit Cx43 (Sigma, 1:1000) and Mouse α-actinin (Sigma, 1:1600). Protein density was measured using the NIH ImageJ software as previously described.\textsuperscript{27} For details see Online Data Supplement.

Statistical Analysis
Values are expressed as means±SEM. Hypothesis testing was carried out using an unpaired Student t test and χ\textsuperscript{2} analysis with Yates correction. A value of P<0.05 was considered statistically significant.

Results
Transmural Dispersion of Repolarization in Human LV
Figure 2 shows maps of transmural activation and APD distribution contour maps from nonfailing (heart no. 3) (A) and failing (heart no. 4) (B) human hearts are shown at pacing CL=2000 ms. Optical fields of view are denoted by rectangles on corresponding photos. Endocardial pacing sites are marked. Color scales represent the activation time and APD in corresponding maps. Selected subepicardial (EPI), midmyocardial (MID), and subendocardial (ENDO) APs are superimposed and demonstrated for each heart.
The maps demonstrated the homogeneous activation patterns without conduction delays or blocks indicating the quality of preparation perfusion, tissue condition, and robust cell-cell coupling. Transmural activation time was in agreement with Durrer et al. Transmural conduction velocity was 41.4 cm/s (n = 10) at 30 bpm.

In nonfailing human LV, we observed significant differences between APD at the subepicardium versus the subendocardium and midmyocardium (Figure 3A), which resulted in a substantial transmural APD gradient (Figure 3D). HF remodeling induced transmurally heterogeneous prolongation of APD, which resulted in a significantly stronger lengthening of APD at the subepicardium than at the subendocardium and midmyocardium (Figure 3B and 3C). The average functional refractory period for fast pacing was 41.4 ± 10.6 ms (n = 10) at 30 bpm.

We found 2 different patterns of transmural APD distribution. The first pattern was observed in 100% of failing hearts and in 40% of nonfailing donor hearts (2 of 5) and was characterized by a smooth decrease in APD from the subepicardium to the subendocardium and midmyocardium (Figure 3A). However, in 60% (3 of 5) nonfailing hearts, we found islands of cells, which were localized in deep subendocardium or midmyocardium and possessed a distinctly long APD compared with the neighboring myocardium. See Online Figure III, with all 5 examples of APD distribution in nonfailing hearts. The average APD values for these islands were calculated in these 3 hearts and presented as a separate group (Max-APD) in Figure 3A and 3B. Examples of APD distribution in nonfailing hearts are presented in Figure 2 (heart no. 4), Figure 5 (heart no. 2), and Online Figure IV (heart nos. 3 and 4).

To describe these islands in detail, we quantified the transmural APD distribution throughout the mapped area. We found a relatively gradual increase in APD from the subepicardium to the subendocardium and midmyocardium (Figure 3D). A representative example is illustrated in Figure 2B. See also Online Figure IV (B), with 2 more examples from the other failing hearts; and Online Tables II and III, with all data for transmural APD distribution in nonfailing and failing hearts.

Figure 3. Summary data for transmural distribution of APD in nonfailing (n = 5) and failing (n = 5) human hearts. A, Transmural APD80 distribution in nonfailing human hearts at different pacing CL. APD80 were calculated for different transmural areas: subepicardium, midmyocardium, subendocardium, and in the region with the longest APDs (Max). *p < 0.05 for Max cells vs subendocardium; #p < 0.05 for subepicardium vs subendocardium. B, Transmural APD distribution in failing human hearts at different pacing CL. C, APD80 for subepicardial (Sub-Epi), midmyocardial (Mid), and subepicardial (Sub-Endo) layers are presented for nonfailing and failing human hearts at 2000- and 1000-ms pacing CL. *p < 0.05 for nonfailing versus failing groups. D, Transmural APD gradients calculated as a difference between the subepicardial and subendocardial APD80 throughout the mapped area are presented for nonfailing and failing hearts at different pacing CL. *p < 0.01, **p < 0.05 for nonfailing vs failing groups.
islands. Figure 4 illustrates this by showing 2 APD distributions in the same LV wedge preparation taken along the different transmural lines, which are perpendicular to the epi- and endocardium. The first distribution (Figure 4B1) shows a gradual APD shortening from the subendocardium to the subepicardium without prominent peaks or sharp gradients. In contrast, the second distribution (Figure 4B2) crosses the deep subendocardial island of delayed repolarization and contains a steep transmural APD gradient with a sharp gradient of APD in the deep subendocardial region marked by the red rectangle on Figure 4B2. Thus, very different transmural APD distribution can be observed depending on the choice of anatomic cross-section or area of subendocardium. Therefore, we conclude that characterization of heterogeneity of transmural gradient by means of APD distribution along a transmural line may be limited and requires the 2D mapping. This ambiguity is especially important for microelectrode studies where the high-density 2D mapping of transmural gradient is impossible.

To characterize these islands further, we established a criterion based on the local APD gradient between them and the neighboring myocardium. Red rectangle in Figure 4B2 marks the distribution of the APD through the Max-APD island. This example demonstrates a sharp transition of APD in the subendocardium characterized by the large local APD gradient (Figure 4C and 4D). We defined Max-APD islands as regions isolated from the neighbors by local APD gradient of >15 ms/mm. Figure 4D shows the vector map of APD gradient in a close-up view of the Max-APD island, and the Figure 4E shows the magnitude map of APD gradient. Figure 4E and 4E demonstrate that the Max-APD island was surrounded by the maximum of the local APD gradient (deep red color). The average size of Max-APD islands was 9.2±2.0 mm² (n=3). All Max-APD islands as well as local APD gradient distributions for other 2 nonfailing human hearts are presented in Online Figure II. In all 3 nonfailing hearts, Max-APD islands were located in the deep subendocardium as presented in Figure 4. The center of these islands was located approximately at 3.4±0.3 mm apart the endocardium (4.1 mm, 3.1 mm, and 3.1 mm for heart nos. 1, 4, and 5, respectively). In one nonfailing heart no. 4, we found 2 islands with prolonged repolarization; one of them was located in the midmyocardium (approximately 9.1 mm apart the endocardium) and did not completely fit by the optical field of view (see Online Figure II); we did not include it in the analysis. Dimensions of Max-APD islands were 2.4±0.6 mm in depth and 4.7±2.2 mm in maximum length on average (n=3).

Progressive shortening of the pacing CL up to the functional refractory period resulted in shortening of APD and decrease of transmural APD gradient (see Figure 3A and 3B). Figure 5 demonstrates the activation and the APD distribution patterns and representative subepicardial, midmyocardial, and subendocardial APDs from 2 typical nonfailing (A) and failing (B) human LV wedge preparations. Data are presented for the nonfailing heart no. 5 and failing heart no. 2. Three pacing CLs from the applied restitution protocol are shown. Progressive decrease of pacing CL resulted in slowing of activation and shortening of APD throughout the wedges. Moreover, APD shortening occurred heterogeneously in the nonfailing hearts and eliminated the transmural APD gradient. For example, during slow pacing at 15 bpm (Figure 5A1) the selected nonfailing wedge exhibited a significant transmoral gradient of 180 ms with prominent APD in midmyocardium areas. However, during fast pacing at 120 bpm (Figure 5C1) the transmural APD gradient decreased nearly in half to 90 ms, and the endocardial and midmyocardial APD became comparable. As described earlier, the failing heart showed reduced transmural APD gradient without a
significant APD difference between the subendocardium and midmyocardium. Thus, in failing hearts, fast pacing also decreased transmural APD gradient in half (from 70 to 35 ms; Figure 5A2 and 5C2, respectively) and fully equalized the endocardial and midmyocardial APD.

Dynamic Properties of Transmural Repolarization

It has been previously shown that the rate dependence of APD is heterogeneously distributed through the mammalian LV.9,10,30 To characterize the dynamic properties of transmural repolarization, we applied the restitution protocol for all tested human hearts. As shown on Figure 5, in the nonfailing hearts, progressive decrease in pacing CL resulted in heterogeneous APD shortening throughout the LV wedge preparation. In agreement with animal studies,8–10,17,30 deceleration-induced prolongation of APD in the nonfailing hearts was much greater in midmyocardial area than in subepicardial and subendocardial regions (Figure 6B). Maps show spatial distributions of APD restitution slopes through the LV free wall in representative nonfailing (Figure 6A, nonheart no. 5) and failing (Figure 6C, failing heart no. 2) human hearts. The color scales represent the value of maximal restitution slope in every single pixel. As indicated by color scales, values of slope for nonfailing heart (A) were significantly higher than for failing heart (C) and significantly exceeded the critical threshold value of one as compared to failing heart where slopes were lower than one throughout the mapped area. Plotted for different regions, the APD restitution curve for midmyocardial area (ie, for Max-APD islands)
revealed a steeper slope as compared to the subepicardium and subendocardium (Figure 6B). In contrast, failing hearts were characterized by approximately equal restitution slopes in the midmyocardium, subepicardium, and subendocardium (Figure 6D). The average APD restitution slopes throughout the mapped area were 1.10±0.09 (n=5) and 0.86±0.12 (n=5) for nonfailing and failing hearts, respectively.

Different restitution kinetics in the Max-APD islands resulted in the presence of electric alternans in these regions. Figure 7 shows the dynamics and anatomic locations of APD alternans (A) and AP amplitude alternans (B) in nonfailing heart no. 5. Four panels in (A) and (B) represent transmural distribution of each type of alternans during progressive decrease of pacing CL. The black areas on maps indicate that the algorithm for APD alternans calculation could not be applied because of incomplete repolarization at this CL or low quality of optical signals. It is evident that alternans of APD started first at CL=260 ms. Figure 7C shows distribution of diastolic intervals as well as corresponding APDs plotted for each pixel during pacing with CL=240 ms. The separation of areas with single colors (red or green) indicates the presence of alternans at this pacing frequency. The representative examples of optical recordings from 3 areas labeled on maps are shown in Figure 7D. APD alternans were
observed in 80% (4 of 5) of nonfailing hearts. We did not observe alternans in the nonfailing heart no. 4. The most predominant location of APD alternans was subendocardium. The pacing CL threshold for APD alternans occurrence in nonfailing hearts was 335±45 ms (n=4). In failing hearts, APD alternans were observed only in heart no. 3, (20% hearts, 1 of 5) during pacing with CL=300 ms. However, in 2 failing hearts (no. 1 and no. 2) we could not induce APD alternans. Therefore, obtained data do not allow us to relate HF remodeling and APD alternans. Additionally, we did not observe any evidence of extra beats and/or arrhythmias in both nonfailing and failing human LV wedges. Neither during slow pacing (CL=4000 ms) nor after termination of fast pacing of restitution protocol there were changes in resting potentials, which could be interpreted as triggered arrhythmia.

Transmural Expression of Cx43

To examine the transmural Cx43 expression in the human heart, optically mapped tissues were studied by immunohistochemistry. Figure 8A represents typical examples of Cx43/α-actinin double immunostained tissues from nonfailing and failing hearts taken from epicardial, midmyocardial, and endocardial locations. The epicardial Cx43 density in both nonfailing and failing hearts was found to be significantly decreased compared with the midmyocardial and endocardial expression (Figure 8B). HF remodeling resulted in a significant decrease of relative transmural expression of Cx43 on the subepicardium. Midmyocardial and subendocardial Cx43 tended to downregulate as well in failing hearts, but the difference with nonfailing hearts did not reach statistical significance.

Discussion

Our study shows that LV remodeling in the human failing heart results in (1) an increase of APD and refractory period, (2) reduction of the maximum slope of APD restitution curve, (3) reduction of local and global transmural APD gradient, (4) occurrence of APD and AP amplitude alternans, and (5) downregulation of subendocardial Cx43 expression.

HF Remodeling

QT-interval prolongation is a well-recognized hallmark of human and experimental HF.1–5 In this study, in failing hearts we also observed prolongation of functional refractory period as well as transmural APD through the LV (Figures 2 and 3). When compared to nonfailing hearts, end-stage failing hearts demonstrated transmurally heterogeneous prolongation of APD, which was evident in a significantly higher lengthening of APD at the subepicardium as compared to the subendocardium and midmyocardium (Figure 3). As a result, the total transmural APD gradient between the subendocardium and subepicardium was significantly decreased during HF (Figure 3D) as also shown in examples in Figures 2A and 5A2 (see also Online Figure IV, B). Interestingly, reduction of transmural APD gradient in LV is contrary to animal models data demonstrated an enhanced transmural heterogeneity in canine failing hearts.4 It should be noted that all animal data were presented from the 4 to 6 weeks rapid pacing model of HF. In contrast, in our study we used human hearts from patients with the end-stage HF, which was progressing over long time (see Online Table IV). It may appear that our findings contradict general consensus on overall increase in dispersion of repolarization in failing hearts. However, we believe it is not so. It is known that both structural and ionic remodeling contribute to increase in dispersion of repolarization. In our study we excluded structural remodeling as a factor by selecting wedges without prominent scarring and fibrosis. In addition, we focused only on midwall transmural myocardium, although there is a possibility of apical-basal APD gradient, which has been demonstrated in several species.31 Finally, only one transmural section was mapped in each human heart and therefore we cannot exclude the possibility that other regions might have APD heterogeneity.

Our results are in agreement with observation of Taggart at al.16 from 21 patients (44 to 84 years old) with different anamnesis. In that study, the physiologically significant transmural gradient within the ventricular wall was absent even during both pronounced bradycardia and early ischemia.16 These changes between early and end-stage HF are likely attributable to remodeling in ion channel and calcium handling protein expression.2,3 In the current study, we have not investigated these changes, and would abstain from unsubstantiated speculations. However, we are in the process of
mapping transmural differences in mRNA and protein expression, which will be reported in a follow-up study.

M Cells

We provide for the first time experimental evidence in support of existence in the nonfailing human heart of a distinct population of subendocardial cells with significantly delayed repolarization as compared to the neighboring myocardium (Figure 4). These isolated islands are characterized by steep local APD gradient (Figure 4) and different restitution properties (Figure 6). The observed islands of cells with significantly prolonged repolarization appeared to be similar to a midmyocardial population, known as M cells, which was observed in several animal models.4,7,12,17 However, in contrast to some reports from animal models, we did not observe contiguous layers of cells in the midmyocardium or deep subepicardium which were referred to the M-cell population.13,30 In contrast, we found the isolated islands of delayed repolarization, in agreement with canine data reported by Akar et al.7 Interestingly, in contrast to nonfailing human hearts, failing hearts demonstrated a relatively flat APD distribution throughout the LV wall without prominent peaks (Figure 5), which could be referred to as M-cell islands.

The original definition of M cells, a distinctive class of midmyocardial cells having exceedingly long APD, when activated at long CLs characteristically different and separated from the subepicardial and subendocardial myocytes,9,30 was based on observations with microelectrode recordings at selected sites. Although M cells were defined, the criteria for separating from other cell types have been less clear. In addition, the differences in the APDs between the M cells and surrounding subendocardium were much smaller in intact canine ventricular wall compared with isolated cells or tissues18,32 as also observed in our study in humans (Figures 4 and 5). Yan et al33 reported that the difference between the above mean APDs (15 ms) was actually smaller than their standard deviations (25 and 21 ms). Such a relatively small difference in APD may not be easily detectable, especially when it is compared with APDs in all mapping sites in the M-cell layer and in the surrounding subendocardium, instead of between small numbers of microelectrode recordings. Alternatively, M cells were also defined as nonepicardial and nonendocardial cells with the longest (10th percentile) APDs.7 This definition is difficult to interpret, because top 10% APDs can always be found even in cases with no statistically significant difference.

In the present study, we proposed a new definition, which is based on the local APD gradient between the islands of delayed repolarization (M cells) and the neighboring myocardium (Figure 4). When studied in isolation, cells could be compared based on their AP morphologies, resting potential and amplitude values. However, being incorporated in the connected myocardium, both their repolarization and restitution properties are dependent on both intrinsic cellular properties (ie, time-dependent ionic currents that govern cellular repolarization) and also extrinsic factors such as propagation32 and cell-to-cell coupling.34,35 Thus, repolarization of a cardiac cell incorporated into the well-coupled myocardium is significantly determined by transmembrane voltage gradients from neighboring cells that either increase or decrease APD.36 Based on our definition, which takes into accounts both intrinsic cellular properties and extrinsic electrotonic coupling, we found the evidence of the isolated islands of delayed repolarization (M cells) in 3 of 5 nonfailing human hearts and no evidence of these islands in failing hearts. Therefore, we suggest that characterization of heterogeneity of transmural gradient and M cells by means of APD distribution along a transmural line may be inappropriate and requires 2D mapping. Moreover, this characterization could lead to the contradictions observed between in vivo and in vitro studies.19

Relationship Between Cx43 and Repolarization

It is presumed that normal cell-to-cell coupling through gap junction would attenuate heterogeneities between different transmural layers in syncytial preparations.15,35–37 The resulting current flow between neighboring cells with different intrinsic repolarization time will tend to delay recovery in cells with an intrinsically short APD and will delay recovery in cells with intrinsically short APD).35,37 Therefore, it has been suggested that intercellular coupling through gap junctions is an important mechanism responsible for maintaining electrophysiological heterogeneities between transmural muscle layers. Experimental evidence supports this hypothesis. Thus, it has been shown in the canine LV that subepicardial expression of Cx43, the principal gap junction protein found in ventricular myocardium, is reduced compared to midmyocardial and subendoocardial layers.6 Moreover, this Cx43 downregulation is well correlated with shorter APD at the subepicardium. Our results in the human heart revealed the same transmural pattern on Cx43 expression through the LV wall as reported by Poelzing et al20 in the canine.

HF remodeling was reported to be associated with significant reduction of Cx43 expression. Such cellular uncoupling has been observed in animal models of HF.15 We have expected to observe similar changes in the human heart. Accordingly, in present study, we found a significant decrease of relative transmural expression of Cx43 in failing hearts (Figure 8), especially in the subepicardium. One would expect that cellular uncoupling caused by HF induced Cx43 downregulation may unmask the intrinsic differences in APD and reveal the distinct subpopulations of cells, which can be referred to the M cells.15 However, in contrast to animal models, we found that in humans HF resulted in reduction of local (Figure 4D) and global (Figure 3D) transmural APD gradients. It appears that the observed reduction in cell-to-cell coupling does not correlate with concomitant decrease in dispersion of repolarization, as compared to normal well-coupled myocardium. Thus, alternative mechanisms have to be considered, such as transmural heterogeneities of ion channel expression.8 Our future studies will focus on this hypothesis.
Limitations

There are several limitations in our study. Firstly, the study was conducted in end-stage failing and nonfailing human hearts, which were considered control. However, these “control” hearts were not from entirely healthy donors (see Online Table V) and 2 of them had early-stage hypertrophy and coronary disease. Thus, we cannot fully extend these findings to healthy human hearts. However, all nonfailing hearts were characterized as hearts without history of HF in anamnesis. Cardiac resuscitation therapy could also affect donors’ heart electrophysiology. The nonfailing heart no. 5 was obtained from the patient who died from the overdose of Tylenol which could affect on the cardiac electrophysiology and induced more distinguished islands of M cell in this heart (Figures 3 and 5 through 7). Nevertheless, we did not observe any significant deviations in nonfailing heart groups. Therefore, it seems possible to compare failing hearts with nonfailing donor hearts as 2 separate groups with different etiology.

Secondly, during the current study, we had a limited number of human heart preparations. Only 5 hearts with heterogeneous disease etiologies were tested in each group. More human hearts have to be tested based on their type of cardiomyopathy. The presented results should be very carefully used when extrapolated on the other types of cardiac disease.

Thirdly, only the posterior-lateral LV ventricular wall was sampled. It is possible that other regions had different repolarization heterogeneity and provided additional islands of delayed repolarization. Our data demonstrate that multiple islands are presented in the nonfailing wedge preparations. However, all in vitro studies are time-limited, and characterization of multiple tissue samples from the same heart is impossible to achieve with limited resources.

Finally, we used isolated wedge preparation and optical mapping, which have their limitations well recognized in similar animal heart preparations, i.e., local heterogeneities of APD could be a consequence of surgery and/or unavoidably altered perfusion pattern at the cut surface. As any coronary perfused transmurally dissected wedge, our preparations could contain damaged regions of myocardium because of laceration of blood vessels. This may result in a moderate degree of regional ischemia, which could affect the presented APD distribution. In the initial experiments, we have excluded 5 hearts form the study, which had clear evidence of ischemia in the field of view. After gaining more experience with the wedge preparations, we have not observed ischemia. Moreover, presence of consistent statistically significant transmural APD gradient at various heart rates in nonfailing hearts indicates that it is likely to be a valid fining.

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Disclosures

None.

References

22. Fedorov VV, Lozinsky IT, Sosunov EA, Anyukhovsky EP, Rosen MR, Balke CW, Efimov IR. Application of blebbistatin as an excitation-


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**Novelty and Significance**

**What Is Known?**

- Numerous animal models have demonstrated that the left ventricular transmural dispersion of repolarization plays an important role in normal physiology and pathophysiology.
- Canine and other animal models of heart failure have showed significant proarrhythmic electrophysiological remodeling, associated with an increased ventricular transmural heterogeneity in cell–cell coupling and in repolarization.
- The clinical relevance of these findings, however, has been challenged given the lack of similar findings in human studies that have been published thus far.

**What New Information Does This Article Contribute?**

- There is a significant transmural gradient of repolarization in the left ventricle of the normal human heart at physiological heart rates.
- Heart failure results in prolongation of action potential duration and overall reduction of transmural gradient of repolarization in the left ventricle.
- Heart failure leads to significant downregulation of connexin 43 in the left ventricular subepicardium.

Sudden cardiac death is a leading cause of mortality in the settings of heart failure. Heart failure induced remodeling of activation and repolarization within the ventricular wall has been shown to play a major role in the onset of ventricular arrhythmias in many animal models. However, contradictory findings attributable to differences in species, genetic backgrounds, and methods of inducing heart failure have led to a lack of consensus on the importance of heterogeneity in human heart failure, which can only be resolved by directly studying human electrophysiology. In our study, we applied for the first time high-resolution optical imaging to characterize the electrophysiology of failing and nonfailing human hearts. Until this study, this methodology has been applied only to animal models. We found that the nonfailing human left ventricle has a significant transmural gradient of repolarization. Paradoxically, heart failure leads to reduction in the transmural gradient of repolarization, a finding contrary to reports in some animal models. Our study provides the first clinical benchmark for the animal and computer models of cardiac electrophysiological remodeling in the setting of heart failure.
SUPPLEMENT MATERIAL

Title: Transmural Dispersion of Repolarization in Failing and Non Failing Human Ventricle

Authors: Alexey V. Glukhov, Ph.D.; Vadim V. Fedorov, Ph.D.; Qing Lou, B.S.; Vinod K. Ravikumar; Paul W. Kalish; Richard B. Schuessler, Ph.D.; Nader Moazami, M.D.; Igor R. Efimov, PhD.

METHODS

Patients groups
Cardiomyopathic failing hearts (n=5) were obtained during transplantation at the Barnes-Jewish Hospital, Washington University in Saint Louis, MO. As a control, we used non-failing donor hearts (n=5), which were rejected for transplantation for various reasons, including age, early stage hypertrophy, atrial fibrillation, and coronary disease. Ejection fraction values were obtained immediately before heart removal from the patient chest and could reflect the effect of patient reanimation. Nevertheless, all non-failing hearts were characterized as hearts without history of heart failure. Donor hearts were provided by the Mid-America Transplant Services (Saint Louis, MO). Online Table I presents clinical characteristics of non-failing human hearts. In hearts #1 and #4, the early stage of hypertrophy was observed which together with the patient age have an effect on the heart electrophysiology. However, we did no observe any deviations in
these hearts compared with another non-failing hearts (data from non-failing heart #4 is presented on Online Figures II - IV). Acute cardiac ischemia as well as a presence of atrial fibrillation in heart #2 were caused by resuscitation of the patient and were not chronical (data from non-failing heart #2 is presented on Online Figures III and IV, and did not differ from another non-failing hearts). Non-failing heart #5 was obtained from the patient who died from the overdose of Tylenol which could affect on the cardiac electrophysiology and induced more distinguished islands of M-cell in this heart (Figures 2, 3, 5-7 and Online Figures II and III).

**Experimental preparation: Quality of perfusion**

We isolated wedges of the human ventricular wall as previously described in canine heart. Briefly, after harvesting, the hearts were immediately perfused through the aorta with a cardioplegic solution. The cardioplegic perfusion washed out the blood and protected the hearts during the subsequent period of wedge isolation. We then isolated a transmural wedge from the posterior-lateral part of left ventricular (LV) free wall. The thickness of the LV was not differ between non-failing and failing wedge preparations (16.6±1.4 mm vs 19.2±1.5 mm, respectively, \( p=0.26 \)). Data from the each heart are presented in Online Table III. The preparation was dissected several centimeters below the base of the ventricles and extended about 3 cm towards the apex. All preparations were dissected approximately from the same area of the hearts with the regard for the quality of perfusion.
Before dissection, the quality of perfusion was verified by injection of Methylene Blue dye (Sigma, St. Louis, MO). Based on the blue color in areas supplied by solution, the appropriate preparation was selected. Each wedge contained a section of coronary artery (diameter: ≥1 mm) along its length, which was cannulated with the flexible plastic cannula custom made for these experiments. The quality of perfusion of the dissected preparation was also verified by injection of Methylene Blue dye. Poorly perfused tissue was trimmed from the wedges. Major arterial leaks in the wedges were ligated with silk suture. The isolated tissues were mounted in a warm chamber with the dissected exposed transmural surface up, facing the optical apparatus. The preparation was both superfused and coronary perfused Tyrode solution. We maintained 37°C and an arterial pressure of 60–70 mmHg. The preparation was fully immersed in the perfusion efflux, which assured appropriate superfusion.

After 20-30 minutes of washout, gradual warming after cold cardioplegia to 37°C, tissue recovery, and stabilization, the wedges were stained with 4 µM of membrane potential-sensitive fluorescent dye di-4-ANEPPS (Molecular Probes, Eugene, OR). The dye staining was possible only in well-perfused areas of the preparation which was an additional verification of quality of adequate tissue perfusion. The optical field of view was selected so as to avoid the areas with low fluorescent signal.
Data processing and analyzing

A custom-made Matlab-based computer program was used to analyze APs offline. First, the signals were filtered using the low-pass Butterworth filter at 50 Hz. Activation maps were constructed from activation times, which were determined from the dV/dt_{max} in each channel. Finally, AP duration was calculated as time difference between the activation time (dV/dt_{max}) and 80% of repolarization (APD80).

Online Figure IV represents four examples of activation and APD distribution contour maps. Conduction velocity was calculated as the maximal velocity in the endocardium-epicardium direction. Because of the endocardial pacing, it was not possible to quantify conduction velocity in transversal direction of excitation propagation as well as its anisotropy. We also did not test an effect of different pacing sites on the APD distribution pattern and transmural conduction as proposed in other studies.

APD for sub-endocardial and sub-epicardial layers were calculated by the averaging of maximal values of the first several pixels (before any significant APD changes) from the each side respectively to resolve the presence of M-cell islands. Transmural APD gradient was calculated as the difference between maximal and minimal values of APD through the transmural cross-section of LV (Online Figure IV).
Local APD gradient was measured by \( \left( \left( \frac{d(APD)}{dx} \right)^2 + \left( \frac{d(APD)}{dy} \right)^2 \right)^{\frac{1}{2}} \), where \( x \) and \( y \) were the directions of rows and columns of pixels. \( \frac{d(APD)}{dx} \) or \( \frac{d(APD)}{dy} \) was estimated by APD difference of neighboring pixels over the length of a pixel. To reduce the sensitivity to noise, APD map was first filtered by a 3 by 3 averaging filter before the estimation. To estimate the size and area of islands with prolonged APD (shown in brown color on panels C of **Online Figure II**), we used the following steps. First, region with APD within the top 30% range was identified. That is, APD cut-off threshold was calculated by \([APD_{max} - 0.3 \times (APD_{max} - APD_{min})]\). The qualified areas were marked by dotted lines on **Online Figure II**. Second, local APD gradient distributions were calculated in these areas (see **Online Figure II B**). Threshold of 15 mm/ms was used to identify the high-APD-gradient boundary around the island with prolonged repolarization (dark red color on panels B). After then, maximum APD within this local APD gradient boundary was identified and then used as a threshold for island with prolonged repolarization. APD threshold was individual for each particular island (see **Online Figure II C**). The area of the island was then calculated as a product of number of pixels within the island and the size of a single pixel (see **Online Figure II C**).

**Microelectrode recordings: Effect of Blebbistatin**

To immobilize the preparation, we used the excitation-contraction uncoupler Blebbistatin (BB, 10 µM).\(^5\-7\) The glass microelectrode technique was used to
validate optical action potentials and effect of Blebbistatin on action potential morphology in the isolated coronary-perfused preparation (Online Figure I A) as described earlier. Transmembrane potentials were recorded at 5 kHz by conventional glass microelectrodes filled with 3 M KCl-filled glass microelectrodes with 15- to 25 MΩ resistance. As shown in Online Figure I B, in the failing human LV wedge preparation, BB did not change AP morphology and only insignificantly prolonged subendocardial APD from 323±24 ms to 345±18 ms (n=2), which confirmed our previous results in animals.6 Because we do not have data from non-failing hearts, we can not exclude a different effect of BB in these hearts. However, based on the ECG data, it seems unlikely.

Histology
Histology and immunofluorescence experiments were performed as previously described.5 After optical mapping experiments, human LV wedge preparations (n=10) were perfused with 3.7% formaldehyde for 5 minutes and left in the solution overnight. Then, the preparations were transferred to 20% sucrose for two days before the tissue was frozen. Wedge preparations were embedded in Tissue-Tek OCT compound (Histo Prep; Fisher Scientific, Fairlawn, NJ, USA), frozen in isopentane, and cryosectioned parallel to the epicardium. Tissue sections were mounted on Superfrost Plus glass slides (Fisher Scientific, Pittsburgh, PA) and maintained at −80°C until use. Sets of 16 µm cryosections were cut using a cryostat (Minitome Plus, TBS, Durham NC). Three different regions from the human LV preparation were sectioned parallel to the epicardial
surface: subepicardium (200-600 μm after the connective and epithelial tissue), mid-myocardium (at the middle of the depth), and subendocardium (200-600 μm after the connective and epithelial tissue and papillary muscle beginning). Sections were stained for histology with Masson's trichrome (International Medical Equipment, San Marcos, CA, USA). The examples of histological staining for subepicardial, midmyocardial, and subendocardial sections are presented on Online Figure V A.

**Immunohistochemistry**

In addition to histology, we used neighboring sister-sections for immunohistochemistry. Immunolabeling was carried out as described previously.\(^8\),\(^9\) Sections were stained with commercially available antibodies: Rabbit Cx43 (Sigma, 1:1000) and Mouse α-actinin (Sigma, 1:1600). Primary stains were applied overnight and then secondary antibodies were applied for 2.5 hours. Protein density was measured using the NIH ImageJ software as previously described.\(^5\) Briefly, to quantitatively analyze the amount of Cx43 expression with respect to α-actinin, we obtained 20x images from the Nikon Confocal C1 microscope of 5 randomly selected fields of view in each area of myocardium: epi-, mid-, and endocardium. For each image, we used the ImageJ software with a thresholding macro to determine the ratio of connexin 43 to α-actinin.
Sets of 16 µm cryosections were cut using a cryostat. Three different regions from the human left ventricle preparation were sectioned parallel to the epicardial surface: subepicardium, midmyocardium, and subendocardium.

At least 3 different sections, from subepicardium, midmyocardium, and subendocardium regions, were double-stained for Cx43 and α-actinin with commercially-available antibodies. The following primary antibodies were applied overnight at 4°C: rabbit anti-Cx43 (Sigma, 1:1000) and mouse anti–α-actinin (sarcomere specific, Sigma, 1:1600). The following secondary antibodies were applied for 2 hrs at room temperature: Alexa Fluor 488 goat anti-rabbit IgG (Molecular Probes, 1:1000) and Alexa Fluor 555 goat anti-mouse IgG1 (Molecular Probes, 1:1000).

Protein density was measured using the ImageJ software (National Institute of Health) as previously described. Briefly, to quantitatively analyze the amount of Cx43 expression with respect to α-actinin, we obtained 20x images from the Nikon Confocal C1 microscope of 5 randomly selected fields of view in each area of myocardium: subepi-, mid-, and subendocardium. For each image, we used the ImageJ software with a thresholding macro to determine the ratio of connexin 43 to α-actinin. A custom ImageJ Threshold macro was used to quantitatively analyze protein densities and ratios on immunohistochemistry slides fluorescently labeled with red and green secondary antibodies. Using the ImageJ interface, this program first split a fluorescently stained image into its three RGB
color channels. The user must manually identify in each RGB color channel signal and tissue threshold numbers (an intensity value between 0-255) to be used for each image selection. All pixels with intensity values equal to or above the threshold input values were included in the calculations. For this study, we set our signal threshold value based on ImageJ’s AutoThreshold function as described previously.\(^5\) Using ImageJ’s Threshold function, we identified the threshold values which, when best combined, represented the entire tissue area. The tissue threshold input must be smaller than the signal threshold input value.

The threshold macro permitted the user to define the image selection(s) using three modes: Rectangle, ROI, or Rectangle ROI. Rectangle mode allowed the user to define a particular rectangular region of an image. It then broke up the output measurements into one row of sub-rectangular image-selections within this region. In ROI (Region of Interest) mode, the user could use ImageJ’s selection tools (e.g. polygon and ellipse) and functions (AnalyzeParticle) to manually define particular image selection regions. The Rectangle ROI combined the Rectangle and ROI modes to create a pixilated overview of an entire image selection.

The threshold macro measures and outputs RGB Signal Area, Tissue Area, RGB Signal density, and RGB Signal Ratio values in each of the image selections. As previously described, each color channel’s signal and tissue area were measured based off user input values. Signal density values were defined as a function of
each color channel's signal area divided by tissue area. RGB Signal Ratios were then calculated using each color channel’s signal area.

**Statistics**

Comparisons between groups of data were performed using a t-test for non-paired measurements and nonparametric Kolmogorov-Smirnov test. A value of p<0.05 was considered statistically significant.
Reference List


Figure and Table Legends

**Online Table I.** Patients’ information. Group 1: Failing hearts.

**Online Table II.** Patients’ information. Group 2: Non-Failing hearts.

**Online Table III.** The thickness of the LV wedge preparation (in mm).

**Online Table IV.** Transmural distribution of APD80% in non-failing (n=5) and failing (n=5) human hearts at different pacing cycle length. The first part of the table represents APD80% values in different transmural areas: sub-epicardium, midmyocardium, sub-endocardium and in region with the longest APDs (Max). The second part of the table contains p-values for differences between non-failing and failing groups. Statistically significant changes are highlighted by red color.

**Online Table V.** P-values for transmural differences of APD80% in failing (n=5) and non-failing (n=5) human hearts at different pacing cycle length. Statistically significant changes are highlighted by red color.

**Online Figure I.** Effect of the excitation-contraction uncoupler Blebbistatin in human ventricle. The microelectrode recordings were used to validate the effect of Blebbistatin (10 μM) in the human left ventricle. The photo of the coronary perfused wedge
preparation in experimental the chamber is shown on A. Blebbistatin did not change action potential morphology (B), which confirms our previous results in animals. The microelectrode recordings are presented from the failing heart #1.

**Online Figure II.** Location of Max-APD islands.

Three examples from non-failing human hearts, where Max-APD islands were found, are presented. Data are shown from heart #1, #4, and #5 during pacing with cycle length 2,000 ms. Dotted line shows the region with APD within the top 30% range where local APD gradient threshold was applied and Max-APD islands were defined. To estimate size and area of Max-APD islands (brown areas on panels C), we used criteria based on the local APD gradient (panels B) with the threshold of 15 mm/ms. Maximum APD within this local APD gradient boundary line was identified and then used as a threshold for island with prolonged repolarization (shown on panels C for each example). Solid line on panel C shows the tissue border. It should be noticed that every heart had a individual size of the optical field of view which is shown by corresponding scales.

**Online Figure III.** Transmural APD distribution in all non-failing human hearts used in the study.

**Online Figure IV.** Transmural APD gradient in non-failing (A) and failing (B) human left ventricle wedge preparations.
Data are presented for non-failing hearts #2 and #4, and for failing hearts #3 and #4. Examples of activation and APD distribution patterns are plotted at CL=2000 ms. Next to APD distribution contour maps, corresponding examples of transmural APD distribution through the marked cross sections are presented for each heart. Time scales for APD distribution contour maps are identical for all hearts and amount to 130 ms with different range for the first heart (from 320 ms to 450 ms) against another hearts (from 410 ms to 540 ms). Transmural APD distributions are plotted in the same time range as corresponding APD distribution contour maps. Red dotted lines represent the maximum and minimum values of APD distribution through the transmural cross section. As shown on plots, local APD gradient was significantly bigger for non-failing hearts compared with failing hearts.

**Online Figure V.** Masson Trichrome staining of transmural sections from the human left ventricle.

**A:** Sample images from non-failing and failing human hearts taken from the subepicardium, midmyocardium, and subendocardium using a 10x magnification are presented. **B:** Average ratio of cardiac tissue to connective tissue throughout the hearts.
Online Table I. Patients’ information. Group 1: Failing hearts.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Device-base therapy</th>
<th>Pharmacological treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>64</td>
<td>Nonischemic cardiomyopathy</td>
<td>AICD</td>
<td>Heparin, Beta, Statin, Inotrope</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>37</td>
<td>Dilated cardiomyopathy</td>
<td>AICD</td>
<td>ACE, ARB, Coumadin</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>28</td>
<td>Idiopathic cardiomyopathy</td>
<td>AICD, Biven PM</td>
<td>ACE, ARB, Coumadin, Inotropes</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>35</td>
<td>Restrictive cardiomyopathy</td>
<td>AICD, PM</td>
<td>ACE, ARB, Coumadin, Inotropes</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>61</td>
<td>Ischemic cardiomyopathy</td>
<td>AICD, PM</td>
<td>Beta, ACE, ARB, Coumadin, Inotropes</td>
</tr>
</tbody>
</table>
## Online Table II. Patients’ information. Group 2: Non-Failing hearts.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Blood Pressure</th>
<th>Heart Rhythm, bpm</th>
<th>Ejection Fraction, %</th>
<th>Anamnesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>55</td>
<td>Death from stroke</td>
<td>112/56</td>
<td>101</td>
<td>20</td>
<td>RV function normal; Severely depressed LV systolic function; Dilated LV.</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>19</td>
<td>Brain Death from accident</td>
<td>145/87</td>
<td>130</td>
<td>45</td>
<td>Normal size heart; Acute cardiac Ischemia 32%; Atrial Fibrillation.</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>33</td>
<td>Death from Brain damage</td>
<td>120/80</td>
<td>94-140</td>
<td>25</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>68</td>
<td>Death from stroke after surgery</td>
<td>110/60</td>
<td>70</td>
<td>65</td>
<td>Mild LV Hyperdroyphy; History: Prostate cancer</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>20</td>
<td>Death from overdose of Tylenol</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Online Table III. The thickness of the LV wedge preparation (in mm).

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Non-Failing</th>
<th>Failing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.2</td>
<td>20.8</td>
</tr>
<tr>
<td>2</td>
<td>21.9</td>
<td>19.1</td>
</tr>
<tr>
<td>3</td>
<td>14.2</td>
<td>23.2</td>
</tr>
<tr>
<td>4</td>
<td>15.7</td>
<td>14.1</td>
</tr>
<tr>
<td>5</td>
<td>17.2</td>
<td>18.6</td>
</tr>
</tbody>
</table>

Average ± SEM: 16.6 ± 1.4 19.2 ± 1.5  \( p=0.26 \)
Online Table IV. Transmural distribution of APD80% in non-failing (n=5) and failing (n=5) human hearts at different pacing cycle length.

<table>
<thead>
<tr>
<th>APD80% (ms)</th>
<th>4,000 ms</th>
<th>2,000 ms</th>
<th>1,000 ms</th>
<th>750 ms</th>
<th>500 ms</th>
<th>300 ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 bpm</td>
<td>30 bpm</td>
<td>60 bpm</td>
<td>80 bpm</td>
<td>120 bpm</td>
<td>200 bpm</td>
</tr>
<tr>
<td><strong>Sub-Endo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Failing</td>
<td>510 ± 22</td>
<td>494 ± 22</td>
<td>437 ± 18</td>
<td>383 ± 21</td>
<td>316 ± 15</td>
<td>226 ± 9</td>
</tr>
<tr>
<td>Failing</td>
<td>516 ± 39</td>
<td>506 ± 35</td>
<td>450 ± 35</td>
<td>373 ± 21</td>
<td>305 ± 17</td>
<td>207 ± 10</td>
</tr>
<tr>
<td><strong>Max</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Failing</td>
<td>615 ± 45</td>
<td>537 ± 40</td>
<td>466 ± 25</td>
<td>410 ± 16</td>
<td>330 ± 12</td>
<td>228 ± 3</td>
</tr>
<tr>
<td>Failing</td>
<td>521 ± 30</td>
<td>495 ± 30</td>
<td>455 ± 34</td>
<td>379 ± 20</td>
<td>313 ± 11</td>
<td>215 ± 8</td>
</tr>
<tr>
<td><strong>Mid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Failing</td>
<td>471 ± 21</td>
<td>455 ± 20</td>
<td>405 ± 16</td>
<td>362 ± 20</td>
<td>297 ± 12</td>
<td>216 ± 8</td>
</tr>
<tr>
<td>Failing</td>
<td>507 ± 32</td>
<td>495 ± 29</td>
<td>437 ± 31</td>
<td>367 ± 19</td>
<td>299 ± 13</td>
<td>206 ± 6</td>
</tr>
<tr>
<td><strong>Sub-Epi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Failing</td>
<td>408 ± 21</td>
<td>383 ± 21</td>
<td>350 ± 16</td>
<td>316 ± 16</td>
<td>263 ± 12</td>
<td>196 ± 8</td>
</tr>
<tr>
<td>Failing</td>
<td>482 ± 25</td>
<td>477 ± 22</td>
<td>415 ± 18</td>
<td>357 ± 13</td>
<td>292 ± 9</td>
<td>196 ± 5</td>
</tr>
</tbody>
</table>

**p-values (non-failing vs failing)**

<table>
<thead>
<tr>
<th></th>
<th>Sub-Endo</th>
<th>Max</th>
<th>Mid</th>
<th>Sub-Epi</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>p</strong></td>
<td>0.907</td>
<td>0.192</td>
<td>0.416</td>
<td>0.031</td>
</tr>
<tr>
<td><strong>(non-failing vs failing)</strong></td>
<td>0.779</td>
<td>0.507</td>
<td>0.285</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>0.756</td>
<td>0.834</td>
<td>0.377</td>
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</tr>
<tr>
<td></td>
<td>0.758</td>
<td>0.377</td>
<td>0.865</td>
<td>0.101</td>
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<tr>
<td></td>
<td>0.644</td>
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<td></td>
<td>0.285</td>
<td>0.278</td>
<td>0.470</td>
<td>0.978</td>
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<tr>
<td></td>
<td>0.268</td>
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</table>
Online Table V. P-values for transmural differences of APD80% in failing and non-failing human hearts at different pacing cycle length.

<table>
<thead>
<tr>
<th></th>
<th>Non-Failing Hearts (n=5)</th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>4,000 ms</td>
<td>2,000 ms</td>
<td>1,000 ms</td>
<td>750 ms</td>
<td>500 ms</td>
<td>300 ms</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 bpm</td>
<td>30 bpm</td>
<td>60 bpm</td>
<td>80 bpm</td>
<td>120 bpm</td>
<td>200 bpm</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs Sub-Endo</td>
<td>0.001</td>
<td>0.001</td>
<td>0.002</td>
<td>0.035</td>
<td>0.080</td>
<td>0.432</td>
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<td>0.585</td>
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Online Figure I
Online Figure II

Non-failing heart #1 (CL=2,000 ms)

A. APD distribution

B. APD gradient

C. Max-APD island

Non-failing heart #4 (CL=2,000 ms)

A. APD distribution

B. APD gradient

C. Max-APD island

Non-failing heart #5 (CL=2,000 ms)

A. APD distribution

B. APD gradient

C. Max-APD island
Online Figure III

A. Non-failing heart #1
APD distribution (CL=2,000 ms)

B. Non-failing heart #2
APD distribution (CL=2,000 ms)

C. Non-failing heart #3
APD distribution (CL=2,000 ms)

D. Non-failing heart #4
APD distribution (CL=2,000 ms)

E. Non-failing heart #5
APD distribution (CL=2,000 ms)
A. Non-failing Hearts (CL=2,000 ms)

#2

Activation

APD distribution

#4

Activation

APD distribution

B. Failing Hearts (CL=2,000 ms)

#3

Activation

APD distribution

#4

Activation

APD distribution
Online Figure V

A

Epicardium  Midmyocardium  Endocardium

Falling Heart

Non-Falling Heart

B

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* - p<0.05 vs non-failing