Epicardial Fiber Organization in Swine Right Ventricle and Its Impact on Propagation

Frederick J. Vetter, Stephen B. Simons, Sergey Mironov, Christopher J. Hyatt, Arkady M. Pertsov

Abstract—Fiber organization is important for myocardial excitation and contraction. It can be a major factor in arrhythmogenesis and current distribution during defibrillation shocks. In this study, we report the discovery of a previously undetected thin epicardial layer in swine right ventricle (RV) with distinctly different fiber orientation, which significantly affects epicardial propagation. Experiments were conducted in isolated coronary-perfused right ventricular free wall preparations (n=8) stained with the voltage-sensitive dye di-4-ANEPPS. Optical signals were recorded from the epicardium with a CCD video camera at 800 fps. Preparations were sectioned parallel to the epicardial surface with a resolution of 50 μm or better. To link the histological data with the observed activation patterns, resulting fiber angles were introduced into a 3D computer model to simulate the electrical activation and voltage-dependent optical signals. In all preparations, we detected a thin epicardial layer with almost no depth-dependent fiber rotation. The thickness of this layer (z0) varied from 110 to 930 μm. At the boundary of this layer, we observed an abrupt change in fiber angle by 64±13° followed by a gradual fiber rotation in the underlying layers. In preparations with z0 < 700 μm, optical mapping during epicardial stimulation revealed unusual diamond- and rectangular-shaped activation fronts with two axes of fast conduction. Computer simulations accurately predicted the features of the experimentally recorded activation fronts. The free wall of swine RV has a thin epicardial layer with distinctly different fiber orientation, which can significantly affect propagation and give rise to unusually shaped activation fronts. This is important for understanding electrical propagation in the heart, and further refines the existing knowledge of myocardial fiber architecture. (Circ Res. 2005;96:244-251.)

Key Words: myofiber organization ■ optical mapping ■ propagation

The fiber organization of ventricular myocardium is a significant determinant of both its mechanical function and its electrical propagation. Myocardial fiber organization has been implicated in the mechanisms of ventricular arrhythmias as one of the important factors responsible for the stability of 3D reentrant activity. It is also thought to affect current distribution during defibrillation shocks and thus affect their odds of failure and success. This all explains the persistent interest in structural aspects of myocardial organization and motivates further refining the existing knowledge of myocardial fiber architecture.

The characteristic feature of myocardial fiber organization is the gradual counterclockwise rotation of fibers throughout the heart wall with the total rotation angle from endocardium to epicardium across species ranging from 120° (dog) to 180° (pig). Although in general the dependence of fiber rotation on depth is considered well established, information about fiber organization near the epicardial surface remains scarce.

There are indications in the literature suggesting the existence in some species of a distinct epicardial layer that breaks the pattern of gradual rotation and affects the epicardial and transmural propagation. An indirect indication in favor of such a layer was obtained in our recent optical mapping studies of the epicardial propagation in the pig RV free wall. Instead of slightly distorted elliptical activation fronts, characteristic of anisotropic propagation in 3D tissue with gradual fiber rotation, we observed diamond- and rectangular-shaped fronts with sharp corners. Propagation velocities along the diagonals of a rectangular front were close to 0.6 m/s, a characteristic conduction velocity along myocardial fibers in ventricular myocardium.

These observations led us to hypothesize that the unusual activation patterns are caused by a yet uncharacterized thin epicardial layer with distinctly different fiber orientation. This layer does not follow the trend of gradual fiber rotation and significantly alters the pattern of the epicardial propagation.

The goal of this study was to test this hypothesis by carefully investigating the fiber organization in the epicardial layers and its effect on electrical propagation. We conducted extensive histological studies with improved spatial resolu-
tion in which changes in fiber orientation were reconstructed as a function of wall depth. To achieve superior resolution near the epicardium compared with previous studies,16–18 we collected serial sections at significantly smaller intervals (less than 50 μm). In addition, an automated algorithm was used to detect fiber orientation25 and increase the accuracy of the fiber angle measurements.

To directly relate the histological data to the electrical activation patterns, we conducted optical mapping studies in all tissue samples before fixation and sectioning. To make the quantitative comparison between histological and optical mapping data, we introduced the experimentally derived fiber angles into 3D computer model to simulate optical signals on the epicardial surface of the heart. The simulation results were then compared with the optical mapping data obtained from the same preparation.

The main finding of our studies is the demonstration of previously undetected thin epicardial layer with fiber orientation significantly different from the underlying layers. We obtained compelling evidence from both computer simulations and optical mapping data that this layer has a major impact on epicardial propagation and gives rise to unusual diamond- and rectangular-shaped epicardial activation patterns. The introduction of the experimentally derived histological information into a computer model reproduced activation patterns that were fully consistent with the optical mapping data.

Materials and Methods

Isolated Coronary Perfused Swine RV Preparations

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996). Preparations were perfused with an oxygenated Tyrode solution at 37°C and 80 mm Hg and superfused with the same solution at a rate of 40 mL/min. To the perfusate we added diacetyl-monoxime (DAM, 15 mmol/L) to inhibit contractions and the voltage-sensitive dye di-4-ANEPPS (15 μmol/L). The preparation was paced at a basic cycle length of 500 ms at twice the diastolic threshold within 10 mm of the center of the epicardial surface of the RV free. An expanded Materials and Methods section is presented in the online data supplement available at http://circres.ahajournals.org.

Optical Data Acquisition and Signal Processing

The optical mapping apparatus consisted of a 12-bit digital CCD video camera (Dalsa CA-D1–0128T-STDLD). The area of interest (≈2.2×2.2 cm) was illuminated by a 250W tungsten halogen lamp. The fluorescence was excited at 520±40 nm and recorded at 645±50 nm. The images (64×64 pixels) were acquired at either 500 or 913 frames per second. Optical movies were postprocessed using custom routines written in PV-Wave (Visual Numerics Inc).

Histological Analysis

After acquiring the optical mapping data, the preparation was perfused with 10% buffered formalin. A transmural block of tissue (≈1 cm³) containing the stimulus site was then excised and photographed to record the original orientation and size of the sample for later reconstruction. The small block chosen for histological analysis was excised in the shape of asymmetric trapezoid that uniquely defined its orientation within the intact RV preparation. The center of the trapezoid corresponded to the stimulation site. The sample was then placed between two slices of cork to flatten the epicardial surface during fixation and paraffin embedding.

Serial sections (5 to 10 μm thick) were cut parallel to the epicardial surface and stained with hematoxylin dye. Sections were imaged (261 pixels/mm) with a digital camera (SONY N50 progressive 3CCD). Several images (2.5×1.3 mm) were taken throughout each section to make sure that fiber direction was consistent. The mean fiber orientation in each image was calculated using an intensity gradient algorithm and circular statistics as described by Karlton et al.25 Tissue shrinkage was measured in all sectioned samples. The measured degree of shrinkage was used to rescale the histological data and recover the actual depth of a given section before fixation.

Numerical Modeling

Electrical activity was simulated using the 3D cable equation:

\[ \partial_t V_n = \nabla \cdot \frac{\nabla V_n - I_{ion}/C_m}{D} \]

where \( V_n \) is the transmembrane potential, \( C_m \) is membrane capacitance, \( I_{ion} \) is total ionic current density across the membrane, and \( D \) is the diffusion tensor reflecting the orientation of myocardial fibers inside the tissue. For the formulation of the ionic currents see our earlier publication.28 The components of the diffusivity tensor \( \tilde{D} \) were calculated as described earlier.10,22 The numerical values of the diffusion constants were \( D_{\parallel} = 1 \text{ cm}^2/\text{s} \), and \( D_{\perp} = 1/9 \text{ cm}^2/\text{s} \), corresponding to electrical conduction velocity ratios of 10:3:3 in the longitudinal, transverse, and transmural directions, respectively.

The function \( \theta(z) \) that determines the depth dependence of the components of the diffusivity tensor \( \tilde{D} \) was derived from the measured fiber angles. In the epicardial layer (2 to 3 mm), it was approximated by a variation of the Boltzmann equation:

\[ \theta(z) = \frac{A_2 - A_1}{1 + e^{-z/z_0}} + A_1 + Bz \]

where \( A_1 \) is the fiber orientation after the transition, \( A_2 \) is the fiber orientation at the epicardium, and \( B \) is the slope at the linear portions of the sigmoidal curve. The depth of the transition point is \( z_0 \), where the slope of \( \theta(z) \) is \( \theta'(z) = (A_2 - A_1)/(4d^2)B \). The parameter \( d \) scales the slope at the transition point. Linear fiber rotation was assumed for the remainder of the wall with the total transmural fiber rotation set to 180°.

Simulations were conducted on a 3.2×3.2×0.8 cm rectangular grid. No-flux boundary conditions were imposed at all boundaries.7 Optical action potentials were computed in a 2.2×2.2 cm region on the epicardial surface from the 3D distribution of the transmembrane voltage using the algorithm described previously.28

Results

Histological Analysis

Figure 1 shows the result of the histological analysis for one swine RV preparation. The top panel shows the profile of myocardial fiber angle versus wall depth. The fiber profile had a pronounced sigmoidal shape with almost no fiber rotation for depths less than 290 μm. The fibers then abruptly rotated by approximately 63 degrees relative to the epicardial fibers at the top layer. The middle panel in Figure 1 shows the histological sections acquired at transmural depths of 75 and 550 μm, demonstrating the abrupt change in fiber orientation across the transitional region. At locations deeper into the wall, the rotation continued but at a much slower rate. These data suggest the existence of a distinct epicardial layer with the thickness of approximately 290 μm.

Similar results were obtained in other preparations. All of the transmural fiber profiles had a pronounced sigmoidal shape and were qualitatively similar to that shown in Figure 1. Yet, the depth of the transition from one fiber orientation to another, as well as its steepness, varied from preparation to
The average magnitude of \((A_1 - A_2)\) was approximately 64 degrees. The last two columns show the rates of fiber rotation inside \((S_0)\) and outside \((B)\) the transition region. The values of \(B\) were always significantly smaller than \(S_0\). The values of \(S_0\) followed the same trend as \((A_1 - A_2)\): they tended to be smaller in preparations with larger \(Z_0\).

It should be noted that data presented in Table 1 reflect fiber organization only within the first 1.5 mm from the epicardial surface. Accordingly, values of parameter \(B\) do not represent the rate of fiber rotation in deep layers of the RV. The average rate of fiber rotation across the wall in these experiments was \(39 \pm 22\) degrees/mm, consistent with earlier histological studies.

**Fiber Organization and the Epicardial Activation Patterns**

In all tissue samples before fixation and sectioning, we conducted optical mapping studies that enabled us to relate fiber organization to the epicardial activation patterns. Figure 2, top row, shows sequential snapshots of an expanding epicardial excitation front produced by epicardial point stimulation of the preparation whose fiber organization was illustrated in Figure 1. The first frame was recorded 6 ms after the stimulus, when the front had acquired a pronounced diamond shape with relatively sharp corners and two nearly perpendicular diagonals (dashed lines). The corners of the diamond remained relatively sharp and preserved their orientation as the wave expanded. Conduction velocity measured along the long diagonal (line 1 in Figure 2) was \(0.61\) m/s. Along the short diagonal (line 2), it was smaller (0.41 m/s). However, both velocities were within the range characteristic for propagation velocity along the longitudinal fiber direction.

To relate the activation pattern to the histological data shown in Figure 1, the fiber orientations were superimposed onto the images of the expanding excitation fronts. The dashed lines 1 and 2 in Figure 2 show the fiber orientation in the thin epicardial layer \((z=75 \mu m)\) and in the underlying layer at \(z=550 \mu m\). Note that in the propagating wave, the orientation of the longer diagonal coincided with the surface fiber orientation, whereas the shorter diagonal was closely aligned with the fiber orientation deeper into the ventricular wall.

Similar correlation between the epicardial fiber organization and the shape of the excitation fronts was observed in other preparations. In 7 of 8 preparations, we observed diamond- and rectangular-shaped activation fronts with their diagonals aligned with the fiber orientation in the epicardial and the underlying layer respectively. (An example of a rectangular-shaped activation front is provided in Figure 3.)

As can be seen in Table 2, the angle between diagonals derived from the optical mapping data were rather close to the values of \((A_1 - A_2)\) characterizing the jump in fiber angle at the boundary of the thin epicardial layer and the underlying layers for the majority of the preparations.

As in the example described, in all experiments with diamond- and rectangular-shaped activation fronts, the average value of the conduction velocity along either of the diagonals was very close to the conduction velocity along

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**Figure 1.** Abrupt change in local fiber orientation in swine RV subepicardium. A, Fiber angle as a function of depth, normalized to a zero angle at the epicardium. Abrupt change in fiber angle occurs at a depth of 291 \(\mu m\). Error bars show the standard deviation. B, Portions of two histological sections obtained at depths of 75 \(\mu m\) (1) and 550 \(\mu m\) (2). Solid lines show the local fiber orientation as determined by an automatic algorithm. C, Fit of Equation 2 (solid line) to the measured fiber angles shown in A.
myocardial fibers. Table 3 shows the ratios of conduction velocity along the diagonals. In all cases it was close to 1.

Comparison of histological and optical mapping data in different preparations suggests that the epicardial activation patterns are extremely sensitive to the thickness of the epicardial layer $z_0$ and variations in the transmural fiber rotation, $\theta(z)$. Figure 3A shows three representative examples of various activation patterns in our experiments. The corresponding fiber profiles are illustrated in Figure 3B. Diamond-shaped activation patterns (left column) were observed in preparations with smaller $z_0$ and largest $A_1/A_2$ (profile 1 in Figure 3B). Similar patterns were also recorded in experiments 1 to 3 (see Table 1). Rectangular patterns (middle column) were associated with intermediate $A_1/A_2$ values (profile 2); the classical ellipse pattern (experiment 8) was observed only at largest $z_0$ and smallest $A_1/A_2$ values (profile 3). The rectangular activation pattern was the most common, appearing in four of the eight experiments.

Computer Modeling

Our next step was to confirm quantitatively the role of the fiber organization in the observed unusual shape of activation fronts. The histological data from four representative preparations were incorporated into the computer model to simulate the individual propagating wave fronts observed via optical mapping at the epicardial surface of these preparations.

The bottom row in Figure 2 shows an example of the simulated excitation front using the fitted fiber profile shown in Figure 1. The simulated wave front has a pronounced diamond shape similar to the actual front recorded in the same preparation (compare Figure 2, top row). The origin of the diamond shape can be qualitatively understood when considering a superposition of two conventional elliptical activation fronts, rotated with respect to each other. The first ellipse represents anisotropic propagation in the upper layer, whereas the second ellipse represents anisotropic propagation in the underlying layers (see Discussion for more detail).

**Table 1. Characteristics of Fiber Organization in the Epicardium**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Depth of Transition, $z_0$, $\mu$m</th>
<th>Magnitude of Transition, $A_1-A_2$, degrees</th>
<th>Slope at Transition, $S_0$, degrees/mm</th>
<th>Slope at Plateaus, $B$, degrees/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>110</td>
<td>72</td>
<td>1281</td>
<td>81</td>
</tr>
<tr>
<td>2</td>
<td>291</td>
<td>73</td>
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<td>23</td>
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<td>3</td>
<td>321</td>
<td>89</td>
<td>7194</td>
<td>17</td>
</tr>
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<td>4</td>
<td>525</td>
<td>63</td>
<td>278</td>
<td>45</td>
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<tr>
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<td>556</td>
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<td>584</td>
<td>58</td>
<td>201</td>
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<td>50</td>
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<td>23</td>
</tr>
<tr>
<td>8</td>
<td>928</td>
<td>52</td>
<td>118</td>
<td>23</td>
</tr>
</tbody>
</table>

Mean $\pm$ SD $501\pm256$ $64\pm13$ $1699\pm2578$ $31\pm22$

Parameters derived from $\theta(z)$ fitting using Equation 2.
Computer simulations also confirmed the sensitivity of epicardial activation patterns to the thickness of the epicardial layer and variations in the transmural fiber rotation, \( \theta(z) \). Figure 3A shows examples of computed epicardial activation patterns in three different preparations. Simulations that used experimentally observed variations in the depth and slope of the transition point in the transmural fiber profiles were sufficient to produce dramatically different shapes (bottom row) of epicardial excitation fronts. In all cases, there was excellent agreement between the simulated and experimentally observed activation patterns.

**Discussion**

The most important result of this study is the discovery of a thin epicardial layer in the RV of the pig with a distinctly different fiber orientation and its effect on propagation. We demonstrated that the abrupt fiber rotation occurring at the boundary of this layer produces unusual diamond and rectangular patterns of epicardial activation arising from point stimulation on the epicardial surface. We also showed that variations in the depth of the rapid fiber transition could cause dramatic differences in the shape of the epicardial activation pattern. Simulated activation patterns obtained using experimentally derived fiber angles showed excellent agreement with optical recordings. This provides further support to our histological findings and to our hypothesis that epicardial fiber organization is responsible for the unusual diamond-shaped and rectangular activation patterns observed during epicardial point stimulation.

**Nature of Unusually Shaped Activation Fronts**

As mentioned, the origin of the diamond- and rectangular shapes of the activation fronts can be qualitatively understood when considering a superposition of two conventional elliptical activation fronts, rotated with respect to each other. The first ellipse represents anisotropic propagation in the top layer, whereas the second ellipse represents anisotropic propagation in the underlying layers. The major axis of each ellipse will coincide with the fiber orientation in its respective layer. For a large angle between the axes (80 degrees in our case), such a superposition gives rise to a diamond-like shape of the wave front at the epicardial surface. Indeed, the major axis of each ellipse forms the two diagonals of the diamond, whereas the minor axis of either ellipse is masked by major axis of the other, and cannot be observed.

The fact that the diagonals of the activation front approximately align with the fiber direction in the respective layer provides a simple explanation of rapid propagation along each of the diagonals in Figure 2. It is just a consequence of the fact that the propagation velocity along each of the diagonals represents the longitudinal conduction velocity.

The different length of diagonals (diamond shapes) in some experiments is likely attributable to the delay between the development of a detectable excitation front in the deeper and the top layers. (The stimulus applied at the epicardial surface activates the top layer first, giving rise to a larger ellipse.) The larger differences in the lengths of the diagonals may indicate reduced coupling between the top layer and the underlying layers postulated by Yan and coworkers.\(^{19}\) It is interesting that angles between diagonals derived from the optical mapping data were usually larger than the angles obtained by fitting histological data (the differences are shown in the last column of Table 2). These differences are likely a result of fiber rotation underneath the transition area. It is well established that epicardial optical mapping integrates signals originated from depths significantly exceeding 1 mm.\(^{28}\) Accordingly, fibers located deep in the myocardial wall should contribute to the optical signal and thus increase the observed angle.

Another interesting issue is the variability of structural organization that we observed across specimens (Table 1). It is likely that the thickness of the outer layer, as well as the slope of the abrupt fiber transition, depends on its relative longitudinal position between the apex and base, and its proximity to papillary muscles and the intraventricular septum. A detailed analysis of factors that determine variability of structural organization of the epicardial layer in the pig RV should become a subject of future studies. It is worth noting that for successful prediction of the shape of the activation
fronts, it was necessary to incorporate the unique histological data from the individual preparations into the numerical model. Using data averaged from all the tissue samples would likely not reproduce the unusual epicardial activation patterns that we observed experimentally. This emphasizes the need for precise structural descriptions to accurately predict the electrophysiological effects in individual experimental preparations.

**Left Ventricle Versus Right Ventricle**

Analysis of the literature suggests that the left ventricle (LV) can exhibit a qualitatively similar sigmoidal pattern of fiber rotation that we observed in the RV. Figure 4 compares normalized profiles of fiber angle versus depth for the data we collected in the RV to the pig LV data reported by Streeter and Bassett. Both plots were obtained by averaging data from several preparations, and appear qualitatively very similar: (1) both have an outer layer with almost no fiber rotation, (2) adjacent to the outer layer there is a region of rapid fiber rotation, (3) the rate of fiber rotation decreases toward midmyocardium and then increases again near the endocardium.

On an absolute scale, however, this means the epicardial layer is significantly wider in the LV than in the RV, and thus the maximal rate of fiber rotation in the LV is significantly less than in the RV. The electrophysiological implication of this geometric scaling is that diamond- or rectangular-shaped activation patterns on the surface of the LV are much less likely during epicardial point stimulation. This may explain, in part, why earlier optical mapping studies of the LV have not reported similar observations.

**Generalization to Other Species**

The majority of existing histological studies provide little detail about the fiber orientation at shallow subepicardial depths. However, there is evidence that a thin outer layer with a significantly different fiber orientation may exist in the canine and human LV. In the human LV, Drouin et al reported that the layers of epicardial cells are oriented perpendicular to the subepicardial layers, and hypothesized that the sharp transition was the transmural boundary between the epicardial cells and subepicardial cells (M cells). These studies suggest that the existence of an epicardial layer with distinctly different fiber orientation is not unique to the pig RV and is likely to be present in other species.

**Implications for Arrhythmogenesis and Defibrillation**

The epicardial layer with distinctly different fiber orientation in certain conditions may partially decouple from the rest of the myocardium and become arrhythmogenic. The findings of Drouin et al in the human LV in which such a layer has been detected, show a sharp transition in action potential duration across the layer boundary. This led them to speculate that the electrical coupling between the layers is poor. In the canine LV, Yan et al showed that tissue resistivity rose sharply at the subepicardium, where they identified an abrupt shift in fiber orientation that led them to arrive to a similar conclusion.

Although normally in our experiments we did not observe any indications of electrical uncoupling between the epicardial layer and underlying layers, we did observe a significant degree of electrical uncoupling and reentry during acute global ischemia. The result of one such experiment in the ischemic pig RV is presented in the online data supplement. It shows how the ischemia-induced decoupling allowed the wave propagating in the deeper layer to become reentrant in the thin epicardial layer.

The abrupt fiber rotation attributable to the epicardial layer may affect not only the initiation but also the dynamics of 3D reentry. It has been demonstrated computationally that rapid fiber rotation can affect the shape of 3D reentrant activity and may cause its transition to fibrillation. The organization of myocardial fibers can also affect the distribution of

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**Table 3. Ratio of Conduction Velocities Along the Main Axes in Experiments With Diamond-Shaped and Rectangular Activation Fronts**

<table>
<thead>
<tr>
<th>Exp No.</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio $V_1/V_2$</td>
<td>1.1, 1.5, 1.2, 1, 1.1, 1.2, 1.1, 1.2 ± 0.2</td>
</tr>
</tbody>
</table>

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**Table 2. Comparison Between Histological and Optical Mapping Data**

| Experiment | Change in Fiber Direction, $A_1 - A_2$ | Angle Between Wave Front Diagonals, $A_1 - A_2$ | Difference, $|A_1 - A_2|$ |
|------------|--------------------------------------|-----------------------------------------------|-----------------|
| 1          | 72                                   | 84                                            | -12             |
| 2          | 73                                   | 90                                            | -17             |
| 3          | 89                                   | 91                                            | -2              |
| 4          | 63                                   | 65                                            | -2              |
| 5          | 56                                   | 59                                            | -3              |
| 6          | 58                                   | 67                                            | -9              |
| 7          | 50                                   | 48                                            | 2               |

Mean ± SD $66 ± 13$ | $72 ± 17$ | $-6.1 ± 6.7$ |
so-called “virtual electrodes” during electrical defibrillation, which may determine the outcome of the procedure.\textsuperscript{30,31} It would be interesting to revisit these simulations in light of our findings.

Limitations

The rapid fiber rotation could significantly complicate accurate measurements of the fiber angle in subsurface layers. Accurate measurements require the histological sections to be precisely aligned with the epicardial surface, which we achieved by flattening the epicardial preparations before fixation and careful specimen alignment on the microtome. The precision of alignment was the most critical when the steepness of the transition was very high. Misalignment, however, could be readily identified in transitional sections by the presence of patches with significantly different fiber angles and such sections were excluded from our analysis. This limited the depth resolution of our histological analysis to \(\approx 50\ \mu\text{m}\), yet this resolution is still superior to earlier histological studies.

The limited depth resolution might have affected the accuracy of fitting the maximal slope of the function \(\theta(z)\) and, in part, was likely responsible for its variability. This limitation, however, was a factor only in experiments with the maximal slopes (see Table 1, Experiments 1 to 3). In experiments 4 to 7, where the depth of the transition was deeper and the slope at the transition was less, we did not observe significant variations in fiber angle in transitional sections that would suggest any misalignment. In each of these experiments, we obtained multiple sections from the transitional area, which enabled us to measure the slope with a high degree of confidence.

The histological sections were always smaller than the area of the optically mapped epicardium. We are confident, however, that the epicardial fiber orientation was relatively constant across the mapped surface for two reasons. First, the relative uniformity of fiber orientation on the central RV epicardial surface has been suggested by the fiber reconstructions in the heart of the dog,\textsuperscript{14} rabbit,\textsuperscript{32} and most recently the pig.\textsuperscript{23} Second, we did not detect a change in the direction of fast conduction along the long (epicardial) diagonal in any of the mapped preparations, suggesting that the fiber orientation was uniform on the mapped surface.

For modeling the electrical propagation, we used a monodomain formulation with a simplified description of ionic currents.\textsuperscript{4} We also did not account for the laminar myocardial structure that may affect the properties of the diffusivity tensor.\textsuperscript{4} Despite these simplifying assumptions, the simulation results accurately predicted the observed shapes of the epicardial excitation front. This suggests that subsurface fiber direction is a major factor affecting the geometry of the surface excitation fronts during epicardial point stimulation.

Acknowledgments

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Materials and Methods

Isolated Coronary Perfused Swine RV Preparations

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996). Young pigs (n=8) were heparinized (500 IU, IV) and subsequently anesthetized with sodium pentobarbital (35 mg/kg IV). The heart was quickly removed and Langendorff-perfused with cold (4°C) cardioplegic solution. The right free ventricular wall was excised according to the diagram shown (Supplement Figure 1). The right coronary artery was cannulated. Nonperfused tissue was removed, and the ventricle was mounted in a transparent superfusion chamber. The field of view (~2.2 × 2.2 cm) for the optical maps was centered within 10 mm of the center of the epicardial surface on the excised RV. The preparation was stimulated at the center of the field of view at a basic cycle length of 500 ms at twice the diastolic threshold.
**Supplement Figure 1.** Schematic diagram of the RV preparation relative to the intact heart. The dashed outline shows the boundary of the excised preparation. Solid square: field of view for optical maps; asterisk: stimulation point at the center of the field of view.

**Optical Data Acquisition and Signal Processing**

The optical mapping apparatus consisted of a 12-bit digital CCD video camera (Dalsa CA-D1-0128T-STDL) with a CCTV lens (Computar H1212FI, CBC Corp, Commack, NY). The area of interest (~2.2 × 2.2 cm) was illuminated by a 250 W tungsten halogen lamp. The fluorescence was excited at 520 ± 40 nm and recorded at 620 ± 50 nm. The images (64 × 64 pixels) were acquired at either 800 or 913 frames per second.

Optical movies were post-processed using custom routines written in PV-Wave (Visual Numerics Inc., Houston, TX). To compensate for non-uniformity of staining, local signals were normalized to achieve uniform amplitudes of the action potential throughout the preparation. Signal-to-noise ratio in the movies was improved by spatial and temporal filtering (kernel size of 3-7 pixels).

**Histological Analysis**

After acquiring the optical mapping data, the preparation was removed from the chamber and perfused with 10% buffered formalin. A transmural block of tissue (~1 cm²) containing the stimulus site was then excised and photographed to record the original orientation and size of the sample for later reconstruction. The small block chosen for histological analysis was excised in the shape of asymmetric trapezoid that uniquely defined its orientation within the intact RV preparation. The center of the trapezoid corresponded to the stimulation site. The sample was
then placed between two slices of cork to flatten the epicardial surface during fixation and paraffin embedding (Tissue Tek VIP, Leica).

Serial sections (5-10 µm thick) were cut parallel to the epicardial surface and stained with hematoxylin dye. Sections were examined under 4X magnification and imaged (261 pixels/mm) with a digital camera (SONY N50 progressive 3CCD). Image size was 2.5x1.3 mm. Several images were taken throughout each section to make sure that fiber direction was consistent. The mean fiber orientation in each image was calculated using an intensity gradient algorithm and circular statistics as described by Karlon et al. The parameters of this measurement technique were 20x20 pixel non-overlapping sub-regions and a feature threshold of 1000. Tissue shrinkage was measured in all sectioned samples, and ranged from 31% to 36%, consistent with the data reported in literature. Since uniform shrinkage does not affect the fiber angles, the measured degree of shrinkage was used to rescale the histological data and recover the actual depth of a given section prior to fixation. These procedures enabled the optical mapping data to be directly correlated with the histological data.

**Numerical Modeling**

Electrical activity was simulated using the 3D cable equation:

\[
\partial_t V_m = \nabla \cdot \tilde{D} \nabla V_m - \frac{I_{ion}}{C_m}
\]

where \( V_m \) is the transmembrane potential, \( C_m \) is membrane capacitance, \( I_{ion} \) is total ionic current density across the membrane, and \( \tilde{D} \) is the diffusion tensor reflecting the orientation of myocardial fibers inside the tissue. For the formulation of the ionic currents see our earlier publication. The components of the diffusivity tensor \( \tilde{D} \) were calculated as described earlier.
assuming that the fibers are parallel and uniform in the $x$–$y$ (epicardial) plane but rotate along the $z$ (transmural) direction:

\[
\begin{align*}
D_{xx} &= D_L \cos^2 \theta(z) + D_T \sin^2 \theta(z) \\
D_{yy} &= D_L \sin^2 \theta(z) + D_T \cos^2 \theta(z) \\
D_{xy} &= D_{yx} = (D_L - D_T) \cos \theta(z) \sin \theta(z) \\
D_{zz} &= D_T
\end{align*}
\]

In the above, $D_L$ is the diffusion constant along the fiber direction, and $D_T$ is the transverse diffusion constant. The function $\theta(z)$ is the depth-dependent fiber angle along the $z$ direction, which was determined for each preparation from the histological analyses. The numerical values of the diffusion constants were $D_L = 1 \text{ cm}^2/\text{sec}$, and $D_T = 1/9 \text{ cm}^2/\text{sec}$, corresponding to electrical conduction velocity ratios of 10:3:3 in the longitudinal, transverse, and transmural directions, respectively.

The function $\theta(z)$ in the epicardial layer (2-3 mm) was derived from the measured fiber angles by fitting a variation of the Boltzmann equation:

\[
\theta(z) = \frac{A_2 - A_1}{1 + e^{(z-z_0)/d}} + A_1 + Bz
\]

where $A_1$ is the fiber orientation after the transition, $A_2$ is the fiber orientation at the epicardium, and $B$ is the slope at the linear portions of the sigmoidal curve. The depth of the transition point is $z_0$, where the slope of $\theta(z)$ is $S_0 = (A_1-A_2)/(4d)+B$. The parameter $d$ scales the slope at the transition point. Linear fiber rotation was assumed for the remainder of the wall with the total transmural fiber rotation set to $180^\circ$.

Simulations were conducted on a 3.2 x 3.2 x 0.8 cm rectangular grid. No-flux boundary conditions were imposed at all boundaries\(^8\). To capture the abrupt subepicardial fiber rotation in
the computer model, the transmural resolution of the epicardial portion of the grid (185 x 185 x 58 nodes) was increased with respect to the rest of the grid: $\Delta z = 0.05 \text{ mm}$ versus $\Delta z = 0.15 \text{ mm}$. In the plane of the epicardium, the spatial resolution was the same throughout the entire grid: $\Delta x = \Delta y = 0.175 \text{ mm}$. The node variables on the innermost (last) plane of the fine portion of the grid were updated by a linear interpolation of the variables in the two outermost planes in the coarse portion. Optical action potentials were computed in a 2.2 x 2.2 cm region on the epicardial surface from the three-dimensional distribution of the transmembrane voltage using the algorithm described previously\textsuperscript{6}.

**Preliminary Study of Propagation in the Ischemic Swine RV.**

It is well known that ischemia reduces the excitability of ventricular tissue\textsuperscript{9} and often results in conduction block\textsuperscript{10}. We conducted a preliminary study to investigate the effects of ischemia on propagation in the presence of the thin epicardial layer described in the main text.

**Methods:** The isolated pig RV was prepared as described in the main text. Optical maps were acquired during normal arterial perfusion (control), and during ischemia with a 2-3 minute interval.

**Results:** During control (normoxic) conditions, the wave front demonstrated a diamond-shaped pattern (left panel in Supplement Figure 2), similar to the experiments described in the main text. Middle and left panels show the same preparation after six minutes of ischemia. Instead of forming a diamond (middle panel) the wave front converted to cross-shaped pattern representing a superposition of two ellipses. The longer (vertical) and the shorter (horizontal) ellipses reflect propagation in the epicardial and underlying layers, respectively. The shapes of the ellipses suggest increased anisotropy of propagation due to reduced lateral coupling as well as conduction block between the layers.
Supplement Figure 2: An example of the development of conduction block and reentry due to the thin epicardial layer. *Left:* Control, 10 ms after stimulus. Clearly visible is the diamond shape of the wave front. *Middle and Right:* The same preparation after 6 minutes of ischemia; the numbers on top indicate the time after the stimulus. The wave front acquired a distinct cross pattern. The longer, more vertically oriented elliptical wave front propagated in the thin epicardial layer; the shorter ellipse propagated in the subepicardium. *Right:* The same ischemic region, imaged 32 ms later. Arrows indicate the reentrant wave fronts, one from the thin epicardial layer that entered the subepicardium (arrow 1), and one from the subepicardium that entered the thin epicardial layer (arrow 2).

As both waves continued to expand (right panel), they subsequently created two reentrant wave fronts (arrows 1 and 2). The wave front originating on the epicardial surface propagated across the thin epicardial layer and broke through to the subepicardial layer (arrow 1) where propagation continued in directions perpendicular to the epicardial wave front. In contrast, the wave propagating in the subepicardial layer entered the thin epicardial layer and continued to propagate in directions normal to the originating wave front (arrow 2). These results suggest that the electrical coupling between the thin epicardial layer and the deeper subepicardial layer is sharply reduced during ischemia, and that the abrupt shift in fiber orientation at the boundary between the layers may promote reentry under reduced electrical coupling.

Reference List


