An Image-Based Model of Atrial Muscular Architecture
Effects of Structural Anisotropy on Electrical Activation

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Background—Computer models that capture key features of the heterogeneous myofiber architecture of right and left atria and interatrial septum provide a means of investigating the mechanisms responsible for atrial arrhythmia. The data necessary to implement such models have not previously been available. The aims of this study were to characterize surface geometry and myofiber architecture throughout the atrial chambers and to investigate the effects of this structure on atrial activation.

Methods and Results—Atrial surface geometry and myofiber orientations were reconstructed in 3D at 50×50×50-μm³ resolution from serial images acquired throughout the sheep atrial chambers. Myofiber orientations were determined by Eigen-analysis of the structure tensor. These data have been incorporated into an anatomic model that provides the first quantitative representation of myofiber architecture throughout the atrial chambers. By simulating activation on this 3D structure, we have confirmed the roles of specialized myofiber tracts such as the crista terminalis, pectinate muscles, and the Bachman bundle on the spread of activation from the sinus node. We also demonstrate how the complex myocyte arrangement in the posterior left atrium contributes to activation time dispersion adjacent to the pulmonary veins and increased vulnerability to rhythm disturbance generated by ectopic stimuli originating in the pulmonary vein sleeves.

Conclusions—We have developed a structurally detailed, image-based model of atrial anatomy that provides deeper understanding of the role that myocyte architecture plays in normal and abnormal atrial electric function. (Circ Arrhythm Electrophysiol. 2012;5:361-370.)

Key Words: atrial fibrillation ■ atrial myoarchitecture ■ fiber orientation ■ computer model ■ electric simulation

Atrial arrhythmias are the most common heart rhythm disturbances. Chronic atrial fibrillation (AF) is prevalent in the elderly, increases the risk of stroke, and contributes to mortality in congestive heart failure. There is a strong association between these arrhythmias and the structural remodeling of the atrial chambers that occurs with aging and heart disease. However, despite extensive anatomic and experimental research, the linkage between atrial structure and electric function is not completely understood in the normal heart, let alone in structural heart disease.

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Computer models provide a promising framework for investigating factors that contribute to the initiation and maintenance of reentrant atrial arrhythmias. This view has motivated the development of numerous models of atrial electric function over the past decade. Several of these have incorporated accurate representations of 3D atrial surface geometry and regional differences in atrial electric properties, but none has included detailed descriptions of muscular architecture throughout the atria. Instead, most have assumed that the electric properties of atrial myocardium are isotropic or have incorporated prescribed local anisotropy to account for the role of specialized conduction tracts such as the Bachman bundle (BB) and the pectinate muscles (PMs).

Macroscopic descriptions of atrial muscle fiber organization have been reported in a number of previous studies. High resolution 3D reconstructions of the sinoatrial node (SAN), atrioventricular node (AVN), and right atrial appendage (RAA) have also been presented and it is possible to infer myocyte orientation in each of these. However, intramural myofiber architecture has not been systematically quantified in the atria as has been done for right and left ventricles, using histological techniques and diffusion tensor MRI (DTMRI).

In the current study, we present for the first time an image-based, 3D anatomic model of the sheep atria that...
includes comprehensive descriptions of surface geometry and myocyte organization throughout the atrial chambers. We have used these data to investigate the extent to which myofiber architecture affects the spread of atrial electric activation, with particular emphasis on the posterior left atrium (PLA).

**Methods**

This study was approved by the Animal Ethics Committee of The University of Auckland and conforms to the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85–23). More detailed information on the methods used is provided in the online-only Data Supplement.

**Image Acquisition and Processing**

The atria from a normal sheep heart were fixed at physiological filling pressure and embedded in paraffin wax. Serial surface images were acquired throughout this volume as follows. The upper surface was planed using an Ultramiller, etched to a depth of \( \frac{2}{H11015} \) to \( \frac{2}{H9262} \) \( m \), stained with Toluidine blue, and imaged at \( 8.33-\frac{9262}{H19262} \) \( m \) pixel resolution using a digital camera equipped with a macro lens. This process was repeated in \( 50-\frac{9262}{H19262} \) \( m \) steps. A suite of image processing tools (see online-only Data Supplement Figure I) was used (1) to smooth internal structures and extract tissue boundaries in individual image sections and (2) to smooth the volume image. From these serial images, the atria were reconstructed in 3D with \( 50 \times 50 \times 50-\frac{9262}{H19262} \) \( m^3 \) voxel dimensions and are represented in Figure 1, together with representative image sections, before segmentation.

**Atrial Myofiber Orientation**

Eigen-analysis of the structure tensor constructed from the image volume was used to estimate local myofiber orientation (for detail, see the online-only Data Supplement). Atrial myofiber orientations are initially defined with respect to the imaging coordinate system but are also referred to local coordinate systems where appropriate.

For the imaging coordinate system \( (X, Y, Z) \), the \( X-Y \) plane is near parallel to the atrioventricular (AV) valve plane, whereas the \( Z \) axis lies within the interatrial septum. We define longitudinal as parallel to the \( Z \) direction. Atrial myofiber orientation is specified by defining 2 angles: the inclination angle \( \alpha \) is the projection of the fiber vector onto a plane parallel to the local epicardial surface, measured with respect to the horizontal, whereas the transverse angle \( \beta \) is the projection onto the horizontal plane, with respect to the surface tangent plane (see online-only Data Supplement Figure III).

**Modeling Atrial Activation**

The spread of atrial electric activation was simulated by solving the monodomain reaction-diffusion equation on a 3D voxel-based finite difference grid (\( 100 \times 100 \times 100-\frac{9262}{H19262} \) \( m^3 \) resolution), using the Fenton-Karma activation model. Solutions for isotropic electric properties (conductivity \( =2.4 \) mS) and axially anisotropic electric properties (axial and transverse conductivities \( 9.0 \) mS and \( 0.9 \) mS, respectively) are compared. These values were selected to minimize the difference in predicted activation spread for isotropic and anisotropic cases in regions where myofiber architecture is disordered.

**Results**

**Atrial Myofiber Architecture**

Myofiber arrangement, visualized using fiber-tracking techniques, is rendered on an anterosuperior view of the atria in Figure 2A. This representation is dominated by the subepicardial myofiber organization, but the circumferential orientation of myofibers at the base of the superior vena cava (SVC) and around the pulmonary veins (PV) is evident. Also clear is the BB, which originates near the anterior junction of the SVC and right atrium (RA) and runs leftward parallel to the \( X-Y \) plane across the interatrial groove to the left atrium (LA). This fiber tract continues circumferentially (with re-
spect to the Z-axis) across the top of the LA but gives rise to 2 separate branches. One tracks downward in the LA wall adjacent to the interatrial groove and the other passes along the anterior edge of the left atrial appendage (LAA). Finally, longitudinal myofibers can also be seen immediately below the BB in the interatrial groove between the RA and the LA (see arrow in Figure 2A).

Figure 2B presents myofiber inclination angles mapped onto 6 representative horizontal sections through the atria (see Figure 1B). There are distinct regions of uniformly aligned myofibers throughout the atria. For instance, the BB merges with an extensive band of fibers parallel to it that form the inner half of the anterior LA wall in Figure 2B (2), whereas a second band tracks downward (with respect to the X-Y plane) in the outer LA wall. The orientation of myofibers in much of the interatrial septum and also the septo-pulmonary bundle (SPB) between the right and left inferior PVs (RIPv, LIPv) in the posterior LA wall is uniformly longitudinal. Highly organized tracts such as the crista terminalis (CT) and PMs are also apparent on the endocardial surfaces of the RA wall and the atrial appendages. However, there is no evidence of uniform transmural myofiber rotation (as seen in the ventricles).

Higher-resolution fiber tracking has been used to investigate the microscopic organization of muscle bundles characterized by a high level of myocyte alignment. Typical results are presented in Figure 3. The architecture of a segment from the upper surface of the RAA (7.5×17.5×4 mm; location indicated in Figure 3A) is shown in Figure 3B. Three orthogonal sections indicate the topology of large PM bundles close to their origin along the CT. Fiber pathways are superimposed on these sections and color-coded for inclination angle. As expected, myofibers are strongly aligned with the long axis of the PM bundles. On the other hand, myofiber orientations in the thin atrial wall differ dramatically from PM direction and vary with location. Also, the orientation of myocytes in the large PM bundles is markedly different from those in the CT from which they originate. In Figure 3D through 3F, myocyte organization in the BB is visualized in an anterosuperior segment close to the point at which the BB branches on the left side of the interatrial groove (25×15×7.5 mm; see Figure 3D for location). The ordered myofiber alignment in the BB is clear when fiber tracks are superimposed on orthogonal sections reconstructed within the segment. The BB bifurcates immediately to the left of the interatrial groove; the main stem of the BB continues, while the branch eventually tracks downward toward the AV valve plane.

In Figure 4, we focus on the PLA, with specific emphasis on myofiber architecture at the junction of the PVs and LA. Atrial myocytes in the PLA have an ordered arrangement and wrap circumferentially around the PV sleeves as the veno-atrial junctions are approached. This is evident in Figure 4D, where fiber tracks are rendered on a segment from the right side of the LIPv that also traverses the posterior SPB. The sleeve is thin-walled distal to the veno-atrial junction...
with myofibers predominantly aligned with the PV axis. Sleeve thickness increases abruptly at the veno-atrial junction (from ≈1 mm to ≈4 mm in Figure 4C) and myofibers rotate sharply through ≈90°.

**Effects of Atrial Myofiber Architecture and Geometry on Electric Activation**

To investigate the effects of myofiber architecture on atrial electric activation, we simulated activation spread on the 3D atrial model with isotropic and anisotropic electric properties. The former gives rise to a uniform conduction velocity (CV) of ≈0.75 m/s, whereas the latter produces CVs of ≈1.13 m/s along the fiber direction and ≈0.63 m/s cross-fiber in the RAA segment, where myofibers are uniformly oriented. For regions with heterogeneous myofiber organization, there was very little difference in the activation spread predicted with isotropic and anisotropic electric properties (see online-only Data Supplement Figure V).

In Figure 5, we compare the spread of electric activation from a stimulus site at or near the SAN with isotropic and anisotropic electric properties. Activation spreads uniformly from this site in the isotropic case and is completed ≈116 ms after stimulation. The activation sequence is qualitatively different in the anisotropic case, where preferential propagation pathways are evident in the epicardial activation patterns, and activation was completed within 98 ms. Specific differences include rapid spread from the SAN across the superior RA into the RAA (within 40 ms) and from the RA to the LA, with much of the superior LA activated within 40 to 50 ms. On the other hand, propagation from top to bottom of the atria (particularly the LA) was marginally slower with anisotropic than isotropic electric properties. The role of the CT and PM in the activation of the RAA is indicated by the fact that epicardial activation reflects PM structure. The effects of preferential axial conduction in the BB and to a lesser extent the functional extension of the BB that extends across the anterior surface of the LA are also clear. Finally, activation spreads more rapidly across the LA roof and around the PVs in the anisotropic case.

Further detail on the intramural spread of atrial electric activation is provided in Figure 6, where activation isochrones are superimposed on the 6 representative horizontal
sections in Figure 1B. Activation of endocardial and epicardial surfaces of the atria was relatively synchronous for both isotropic and regionally anisotropic cases. However, the effects in the latter of preferential conduction pathways are evident in the nonuniform distribution of intramural isochrones in associated sections. In the RAA and LAA, contours spread from endocardial to epicardial surfaces in isotropic and anisotropic models. This progression indicates that activation of the atrial appendices is driven by the PMs in both cases but to a much greater extent in the anisotropic model.

The effects of myofiber anisotropy on the spread of atrial electric activation are most marked in the PLA and are shown at higher resolution in Figure 7. In the isotropic case, activation spreads uniformly around the junctions of the right PVs with near synchronous depolarization of the left PV junctions and the lateral margin of the PLA. In comparison, the ordered arrangement of myofibers in the PLA produces strikingly different activation patterns for the anisotropic case. Activation spreads rapidly along the antero-superior margin of the LA (via the BB and its extensions) and across the LA roof and posterior wall between the right and left PVs

Figure 4. Myofiber architecture in posterior left atrium (LA). A, Three-dimensional reconstruction of posterior LA including pulmonary veins. B, Three-dimensional fiber tracks in posterior LA rendered on same posterior view. Gray-scale intensity is selected to emphasize tract orientation and the superior pulmonary bundle (SPB) is indicated. C, Three-dimensional reconstruction of image segment digitally sectioned from the left inferior pulmonary vein (LIPV) adjacent atrial wall as shown in A. The endocardial (lumenal) side is innermost. D, High-resolution fiber tracking for subvolume in C. LSPV indicates left superior pulmonary vein; RSPV, right superior pulmonary vein; and RIPV, right inferior pulmonary vein. Color spectrum as indicated in Figure 3.

Figure 5. Comparison of effects of isotropic and anisotropic electric properties on epicardial spread of electric activation simulated on 3D atrial model incorporating myofiber orientation data. Activation isochrones are rendered on anterosuperior and superior views of the atria in upper and lower panels, respectively. Activation time key for the color map (indicated) same for both. Region in isotropic model not yet activated at 98 ms is indicated in gray. SVC indicates superior vena cava; RSPV, right superior pulmonary vein; LSPV, left superior pulmonary vein; RAA, right atrial appendage; LAA, left atrial appendage; RIPV, right inferior pulmonary vein; and LIPV, left inferior pulmonary vein.
This results in substantial activation time dispersion in the vicinity of the PV junctions and, in particular, adjacent to the LIPV. Latest activation in the lateral edge of the PLA in the anisotropic case occurs in the distal sleeve of the LIPV.

The effects of structural anisotropy on the spread of electric activation from ectopic stimuli in the PLA were investigated by imposing S₂ stimuli adjacent to the PV sleeves after S₁ stimulation near the SAN. The coupling interval was set to the minimum necessary to evoke propagated electric activity (for further detail, see the online-only Data Supplement). Compared with isotropic electric properties, successful propagation was achieved at shorter coupling intervals in the anisotropic case (around 6 ms earlier during repolarization); however, there were substantial initial time delays. The time for activation to reach a line along the SPB, midway between left and right PVs, was 26±10 ms greater for anisotropic than for isotropic electric properties. Initial activation slowing was observed at all 4 PVs, but the range was greatest for the left PVs. A typical example is given in Figure 8C, where activation initially spreads very slowly around the LSPV junction in the myofiber direction but blocks elsewhere (see lines at 40 ms). Of particular interest is the conduction block at this time from the veno-atrial junction into the SPB, transverse to the fiber direction. Tortuous propagation upward and into the SPB, as well as around the LIPV, at 80 ms seeds subsequent coordinated activation of the LA. With isotropic electric properties, activation from the same site was more difficult to achieve initially but spread uniformly across the LA roof and around the left PVs (see Figure 8D), reaching the midline of the SPB 44 ms earlier.

**Discussion**

The spread of electric activation through the atrial chambers of the heart is determined by their geometry and muscular architecture. More comprehensive understanding of the effects of tissue structure on electric function is necessary for effective nonpharmacologic treatment of atrial arrhythmias. In this study, we have developed an image-based model of 3D atrial anatomy, the first as far as we are aware to incorporate a realistic description of myofiber architecture as well as atrial surface geometry. Simulations of electric activation on this structure indicate that the organization of atrial muscle bundles gives rise to significant activation time dispersion in regions such as the PLA. The results of this work support the position taken by Ho et al (2009), who argued that the heterogeneous myoarchitecture of RA and LA and interatrial septum must be taken into account in computer models that seek to investigate mechanisms of atrial arrhythmia.

Computer models are increasingly being used to investigate mechanisms underlying atrial arrhythmias. These include 2D models, monolayer models, models based on simplified morphology, and 3D models. Progressively, computer models have incorporated increased geometric complexity (derived from magnetic resonance or computed tomography image data sets) and differing activation kinetics for different atrial regions. On the other hand, fiber orientation and anisotropic electric properties have to date been assigned manually to prominent fiber bundles only, such as the BB and the right atrial PMs. Our model of atrial structure advances previous work in this area because it captures key features of myofiber architecture throughout the atrial chambers.

**Atrial Myofiber Architecture**

Systematic investigations of myofiber arrangement in left and right ventricles, using serial histological measurements and DTMR, have demonstrated uniform transmural myofiber rotation that varies predictably between regions and is replicated in different species. Comparable, quantitative data have not been available for the atria. The muscular architecture of...
the atria varies across multiple length scales and DTMRI lacks the spatial resolution to capture the complex fibrous architecture of the atrial wall fully. Specialized structures such as the SAN and AVN have been reconstructed in 3D at high spatial resolution 3D,9,10 using serial microscopy. However, our understanding of atrial fiber arrangement and preferential conduction pathways is based on careful anatomic studies by Ho, Anderson, and coworkers,7,8 using dissection, macrophotography, and visual tracing of fiber tracts.

We have estimated myofiber orientation throughout the atria from the 3D structure tensor constructed from the atrial image volume. This well-established image processing technique16 uses methods that are near identical to those used in DTMRI. The intramural fiber orientations obtained in the sheep atria generally match the picture of muscular architecture built up by systematic dissection of human atria.7,8 Myocyte arrangement in the superior atria is predominantly circumferential (with respect to the Z-axis) and is more vertical inferiorly (see Figure 2). The most prominent fiber tracts—CT, PM, and BB—are consistent with previous descriptions of these structures.7,8 Of note are the bifurcations in the BB with minor branches tracking downward adjacent to the RAA and the LAA (see Figure 2A and 3F). Furthermore,
in the upper anterior LA, the BB appears to blend with circumferential myofibers in the inner half of the wall (see Figure 2A and online-only Data Supplement Figure IV, C). Ho et al\textsuperscript{7} described other characteristic myofiber organization in the interatrial septum and LA, including the SPB and septo-atrial bundle. Although it is difficult to replicate the curvilinear viewing planes used in their dissections, the regions of uniformly organized myofibers reported—fibers in or near parallel to the transverse plane in the anterior LA roof, the longitudinal orientation of fibers in the septum and LA wall adjacent to it, and finally, the transverse orientation of subendocardial fibers toward the base of the LA—all appear to be consistent with these earlier descriptions. These findings confirm that the principal features of atrial myoarchitecture are conserved in large mammalian hearts despite differences within and between species.

It is to be expected that our work should be consistent with previous anatomic descriptions.\textsuperscript{7,8} The myofibers tracked within and between species confirm that the principal features of atrial myoarchitecture to be consistent with these earlier descriptions. These findings subendocardial fibers toward the base of the LA—all appear wall adjacent to it, and finally, the transverse orientation of the longitudinal orientation of fibers in the septum and LA or near parallel to the transverse plane in the anterior LA roof, regions of uniformly organized myofibers reported—fibers in or near parallel to the transverse plane in the anterior LA wall adjacent to it, and finally, the transverse orientation of subendocardial fibers toward the base of the LA—all appear to be consistent with these earlier descriptions. These findings confirm that the principal features of atrial myoarchitecture are conserved in large mammalian hearts despite differences within and between species.

Effects of Atrial Myofiber Architecture on Electric Activation

We used our structurally detailed model of atrial anatomy to investigate the effects of myofiber architecture on atrial electric activation. This was done by comparing the spread of electric activation with anisotropic and isotropic electric properties, using a Fenton-Karma activation model\textsuperscript{13} adapted to provide an accurate representation of phase 0 of the atrial action potential (see online-only Data Supplement). We opted for anisotropic electric properties that produce very similar regional activation spread to those seen with isotropic properties in the absence of ordered myofiber organization (see online-only Data Supplement Figure V).

Comparison of activation spread modeled with isotropic and anisotropic electric properties (see Figure 5) demonstrates the impact of myofiber tracts throughout the atria. Activation spreads more rapidly across the endocardial surface of the RA in the anisotropic case, reflecting rapid propagation through the CT and PM (see Figure 5). However, the greatest differences are seen in the LA, with accelerated spread from right to left across the upper anterior atria via the BB and fiber tracts adjacent to it. Most striking is the time course of electric activation in the PLA, where the ordered arrangement of myocytes in the interatrial septum and LA generate preferential propagation from anterior to posterior sides of the LA between right and left PVs (see Figure 7). This gives rise to marked activation time dispersion adjacent to the left PVs.

The activation spread predicted here on the basis of myofiber architecture corresponds very closely to atrial activation patterns mapped in humans,\textsuperscript{17} dogs,\textsuperscript{18,19} and sheep\textsuperscript{20–22} in sinus rhythm or during electric stimulation adjacent to the SAN. In particular, the model replicates normal propagation through the anterior and posterior LA walls during sinus rhythm or stimulation adjacent to the SAN with final activation adjacent to the left inferior PV observed in humans\textsuperscript{17} and dogs.\textsuperscript{18,19} It also reproduces the anisotropic spread of electric activation across the PLA in sinus rhythm in the sheep.\textsuperscript{22}

Over the past decade, it has become evident that the muscular architecture and electric properties of the PLA and PVs play a crucial role in the initiation and maintenance of AF.\textsuperscript{22,23} For ectopic electric activity originating in the PV sleeves, the abrupt changes in myofiber orientation and wall thickness at the junctions of the PVs and LA are thought to give rise to conduction delays and block as a result of current-load mismatch, providing a substrate for reentry.\textsuperscript{22,23} The present study confirms the complexity of myofiber architecture and geometry adjacent to the PV ostia but suggests that the mechanisms responsible for block and slow propagation in the PLA may be more complex than previously thought.

We mimicked the effects of ectopic activity at the PV sleeves by applying S\textsubscript{2} stimuli at the PV junctions at the start of the vulnerable period after S\textsubscript{1} activation from the SAN (see Figure 8C and online-only Data Supplement Figure VII). Successful propagation occurred at shorter coupling intervals with anisotropic electric properties than with isotropic properties but was slow and highly directional. With anisotropic electric properties, transverse current flow is decreased. This reduces current load, enabling propagation to occur earlier in the relative refractory period; however, conduction blocks transverse to the myocyte axis.\textsuperscript{6}

These considerations explain the nonuniform initial spread of electric activation from the S\textsubscript{2} stimulus site in Figure 8C. Activation spreads first along the PV sleeve, but, with the abrupt rotation of myofiber orientation at the PV junction, propagation across the LA roof is blocked and follows the muscle fibers that wrap circumferentially around the left PVs. The slow spread of activation, in this case, during the first 80 ms after S\textsubscript{2} stimulation, is explained in part by the marked repolarization gradients across the inferolateral PLA (Figure 8C, 4 ms). Preferential propagation around the medial side of the left PVs directs activation toward a region of the PLA, where repolarization and CV restitution are initially incomplete. The repolarization gradients reflect the dispersion of activation times in the PLA (see Figure 7), which, as discussed previously, is a result of the complex myofiber pathways adjacent to the BB, around the PVs, and between the PVs (the SPB). In summary, the results of these numeric experiments are consistent with previous views\textsuperscript{22,23} that the complex myofiber architecture at the junction of PLA and PVs provides a substrate for reentry. However, they suggest...
that repolarization time dispersion adjacent to the PVs, which results from the organization of myofiber bundles in the PLA, also plays a role in the initiation of reentrant arrhythmia.

We used spatially uniform isotropic and anisotropic electric properties to model electric activation because it is the most direct way of demonstrating the qualitative effects of atrial myofiber architecture on this process. The electric properties selected gave rise to CVs = 1.13 m/s in the fiber direction and \( \approx 0.63 \) m/s transverse for the anisotropic case in regions where myofibers were uniformly aligned. In the absence of ordered myofiber organization, CV was \( \approx 0.75 \) m/s in all directions, very similar to that observed throughout the atria with isotropic electric properties. Although these CVs lie within the range measured in atrial tissues,\(^2\) electric properties are not spatially uniform even in the normal atria. We probably have overstated the extent of electric anisotropy in working atrial myocardium and understated axial CVs for specialized myofiber tracts such as the BB and CT (see the online-only Data Supplement). This is consistent with the fact that predicted total activation times are somewhat longer than those observed experimentally in the normal atria.\(^{21,22}\) Spatial nonuniformity of atrial electric properties is exacerbated by the structural and electric remodeling that occurs with ageing and many forms of heart disease, and this is thought to increase the risk of atrial rhythm disturbance. For instance, we have demonstrated that abrupt changes in geometry and myofiber orientation at the junction of PV sleeves and PLA give rise slow propagation and activation delays with ectopic activation from the PV sleeves. Almost certainly, heterogeneous atrial electric properties amplify the probability that these factors will give rise to wave break and reentry in patients that are prone to AF.

Future Work

We believe that image-based computer models provide an important platform for better understanding the mechanisms responsible for initiation and maintenance of AF. However, the corollary to this is that the structural remodeling and the spatial variation of atrial electric properties that occur with ageing and heart disease must be incorporated into such models to enable comprehensive analyses of rhythm disturbance to be undertaken. These issues are being addressed in research currently being carried out in our laboratories. The data presented are for the normal sheep heart. Comparable information is being acquired for a sheep heart failure model. These structural data sets will be made available to other researchers via the repository operated by the Cardiac Atlas Project (http://www.cardiacatlas.org).

Conclusions

In this study, we have (1) developed novel techniques that enable surface geometry and myofiber architecture to be characterized throughout the atrial chambers and (2) modeled the spread of electric activation on this structure. We have shown that preferential electric conduction in the atria reflects ordered myocyte arrangement over length scales on the order of millimeters and have confirmed the effects of specialized myofiber tracts such as the CT, PMs, and the BB on the spread of activation from the SAN. We have demonstrated quantitatively, for the first time as far as we are aware, how the arrangement of myocyte bundles such as the BB and the SPB contributes to activation time dispersion in the PLA. Finally, our analysis of structural mechanisms that contribute to increased vulnerability to ectopic stimuli originating from the PV sleeves shows that image-based computer models provide a powerful platform for investigating arrhythmic substrates in the atria.

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Disclosures

None.

References


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**CLINICAL PERSPECTIVE**

Atrial fibrillation (AF) is the most common heart rhythm disturbance, and it is associated with significant morbidity and mortality. The role of the pulmonary veins (PVs) in triggering AF is well established, but the mechanisms by which atrial myofiber architecture contributes to reentry are less clear. To address this issue, we quantified chamber geometry and myofiber orientations throughout the normal atria for the first time and incorporated these data into a 3D computer model of atrial electric activation. We show that specialized conduction tracts and myofiber bundles in septum and posterior left atrium (PLA) give rise to marked activation time dispersion adjacent to the PV junctions in sinus rhythm. We have also demonstrated slow propagation and activation delays when ectopic PV stimuli follow sinus activation. Contributing factors include the abrupt changes in geometry and myofiber orientation at the veno-atrial junctions as well as activation time dispersion in the PLA. The heterogeneous atrial electric properties associated with ageing and structural heart disease almost certainly amplify the probability that these factors will give rise to wave break and reentry. We conclude that anatomically realistic, image-based computer models offer a potentially powerful platform for investigating mechanisms that underlie the initiation and maintenance of reentrant atrial arrhythmias.
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SUPPLEMENTAL MATERIAL

Expanded Methods and Results

Surgery and specimen preparation

A crossbred sheep (45 Kg) was anaesthetized and maintained with isoflurane (2-5%) in oxygen under positive pressure ventilation. The heart was exposed via a sternotomy and the pericardium was divided. Ligatures were placed around the ascending aorta, as well as the superior and inferior vena cavae. These vessels were then occluded and the heart was arrested with a bolus of potassium citrate injected directly into the left ventricle. The heart and lungs were rapidly excised, immersed in cooled (4°C) physiological saline and the coronary circulation was perfused with cardioplegic solution. The pulmonary veins were dissected and ligated as far from the veno-atrial junction as possible and the lungs were removed. The ventricles were then transected to expose the mitral and tricuspid valves, which were trimmed away. The heart was suspended with the atria lowermost and immersed in physiological saline. The atria were slowly filled with warm (50°C) 6% gelatine taking care to ensure that all air was displaced in the process. This was done to prevent them collapsing during fixation. Formalin (3% in phosphate buffer) was perfused through the coronary circulation for 30 minutes and the heart was immersed in this fixative overnight. The heart was placed in a cylindrical plastic container, with the remaining space filled with gelatine, and imaged in a 4.7T MR scanner (Varian Inc., Palo Alto, CA). The heart was extracted from the gelatine and the atria were careful dissected. The atria were supported in an open stainless steel frame and dehydrated in a graded ethanol series over 12 hours. Xylene was used as the intermediate fluid before embedding in paraffin wax (Kendal Paraplast, 56°C melting point) using an automatic processor (TISSUE-TEK VIP 2000, Sakura, Torrance, CA).

Image acquisition

Extended volume surface imaging was used to reconstruct tissue architecture throughout the atria. The block was mounted on a precision 3-axis stage (Aerotech, Pittsburg, PA) and the upper surface was trimmed parallel to the AV valve ring with a variable speed ultramiller (Leica SP2600, Leica Microsystems AG, Wetzlar, Germany) to expose the tissue. The surface was then etched with 25% xylol in 100% ethanol, stained to a depth of ~2 µm with Toluidine blue (0.12% in 1% borax) and imaged at 8.33 µm pixel resolution using an 8 megapixel digital camera fitted with a 65mm macro lens (Canon 1D MkII and Macro Photo MPE, Canon, Tokyo, Japan). Four overlapping images were acquired to cover the area of interest and a composite image was formed using cross-correlation to register the component images. Overlying pixels were averaged. The process of milling, surface staining and imaging was repeated at 50 µm steps throughout the volume. For a more detailed account of these processes, see Gerneke et al, 2007.

Image segmentation and processing

Figure S1 illustrates the steps involved in processing and automatic segmentation of the atrial volume image. The results are compared with a typical 2D composite image prior to processing (Figure S1A). A multi-scale structure filter was used to suppress image noise and background colour due to diffuse lighting of the wax and to enhance the contrast between myofibers and extracellular space in the composite image sections. This approach was initially developed by Frangi and co-workers, who used the multi-scale second order local structure (Hessian matrix) of vascular images to enhance vessel boundaries so that segmentation could be performed as efficiently as possible. Application of the Hessian filter minimized noise and removed background color inside the atrial chambers and adjacent to the epicardial surface. It also minimized the yellow coloration (due to infiltration of wax) of the extracellular space surrounding cells and muscular bundles. A region growing method was then applied to identify and recover tissue areas wrongly suppressed by the Hessian filter due to low stain intensity. The outcome of these processing steps is shown in Figure S1B. Next, sequential dilation and erosion operations were used to connect internal structures and close atrial surface boundaries (Figure S1C). These image processing and segmentation procedures...
were applied to each of the individual sections in the image volume. Subsequently, pixels in the 2D sections were averaged to produce an image volume with isotropic 50×50×50 µm³ voxels. Three dimensional interpolation and smoothing methods were then employed on the processed, down-sampled atrial image stack. Small holes in the 3D surfaces were filled and isolated islands not connected to epicardial or endocardial surfaces were removed. The digital section from the reconstructed segmented atria corresponding to Figure S1A is shown in Figure S1D.

The semi-transparent 3D reconstruction of the atrial walls in Figure S2 provides a sense of both the complex surface geometry of the atria and the organization of the principal conduction tracts. The smooth arrangement of tricuspid and mitral valve rings is evident in the inferior view (Figure S2B).

Extracting fiber orientations

Gradient intensity based methods have been employed widely to extract the orientation of structures in 2D images. The structure tensor method generalizes this approach so that it can be applied to 3D problems. The structure tensor contains gradient information at each voxel in a 3D volume in the form of a matrix. The tensor field was smoothed by convolving it with a Gaussian kernel before applying an eigenvalue decomposition solver to obtain eigenvalues and eigenvectors at each voxel. Local fiber alignment was modeled as the orientation with the least signal variation; this corresponds to the eigenvector paired with the smallest eigenvalue. Finally, the fiber field was further smoothed by averaging fiber orientations in the neighborhood of each voxel.

Analyzing fiber architecture

Atrial myofiber orientation is specified by defining two angles (see Figure S3): the inclination angle α and transverse angle β which are widely used in the field of ventricular fiber analysis. The inclination angle α is the projection of the fiber vector onto a vertical plane parallel to the epicardial or endocardial surface, measured with respect to the horizontal, and has values in the range -90° to 90°. The transverse angle β is the projection of the fiber vector onto the horizontal plane, with respect to the surface tangent plane, and has values between 0° and 180°. The absolute value of the inclination angle |α| is mapped on 2D sections and rendered 3D views to indicate local myofiber orientation. With respect to the imaging coordinate system (X,Y,Z), |α| = 0° is parallel to the horizontal X-Y plane and |α| = 90° is in the Z direction.

Atrial fiber tracts were reconstructed by tracking 3D trajectories defined by myofiber orientations within regions of interest using a simple line interpolation algorithm. The tract was propagated iteratively from a selected seed point, moving an incremental distance in the local myofiber direction (estimated by averaging 8 neighboring orientation vectors) with each cycle.

Atrial myocyte organization in two regions of the anterior left atrium (LA) free wall is displayed at higher resolution in Figure S4. The consistent patterns of transmural fiber rotation seen in right and left ventricles are not observed in the atria and this is obvious here. For the upper segment, there are apparent differences in 3D myofiber organization between inner (subendocardial) and outer (subepicardial) regions. The transmural variation of inclination angle α and transverse angle β defined with respect to the AV valve plane and the local endocardial tangent plane across this segment is presented in Figure S4C. The inclination angle α is near horizontal in the inner half of the wall where it aligns with the BB, but then rotates relatively smoothly from the midwall to around -55° at the epicardial surface. The transverse angle β is uniform in inner and outer regions, but undergoes a sharp transition in the midwall and is oblique with respect to both epicardial and endocardial tangent planes. In contrast, there is much greater local variation in myofiber orientation in the lower LA segment and the 3D reconstruction of fiber tracks in Figure S4F reveals interspersed layers with distinctly different myocyte orientations.

Modeling atrial electrical activation
Electrical activation was simulated on a 3D voxel-based finite difference grid derived from the atrial image volume. Electrical properties were assumed to be axially anisotropic within connected myocyte layers or bundles, with conductivity greatest in the myofiber direction (9 mS) and least transverse to this (0.9 mS). A monodomain reaction-diffusion equation was solved on this structure using a Fenton-Karma\textsuperscript{10} activation model. These solutions are compared with control simulations, in which the electrical properties of the atria were assumed to be uniformly isotropic anywhere (2.4 mS). Activation was simulated in specific atrial subregions at 50×50×50 µm\textsuperscript{3} resolution and in the full atria at 100×100×100 µm\textsuperscript{3} resolution. There was no difference in local CVs estimated at these two resolutions indicating that a spatial resolution of 100×100×100 µm\textsuperscript{3} is sufficient to capture the key features of atrial electrical propagation. The full atrial model (94.9×61.1×32.5 mm\textsuperscript{3}) consists of approximately 2.48×10\textsuperscript{8} spatial points. Simulations were parallelized under MPI and run on an IBM3850 (32 dual thread Intel chips, 256 GB shared memory, Linux operating system). With a time step of 0.01 ms, 1 s of activation could be simulated in less than 8 hours.

It is evident that modeling electrical activity throughout the atrial chambers is computationally demanding even with a resolution of 100×100×100 µm\textsuperscript{3}. This motivated our decision to use the Fenton-Karma\textsuperscript{10} cell model, which replaces the complex ionic current formulations used in biophysically-based atrial activation models (see Courtemanche et al\textsuperscript{11}) with a computationally simpler 3 current scheme. The kinetics of the Fenton-Karma model were adapted to closely match action potential shapes and action potential duration restitution relationships observed in the atria\textsuperscript{12-14}. Further optimization approaches could reduce computational overheads considerably. Atrial tissue occupies a small fraction of total 3D volume (~9%) in our structural model. As proposed by Kharche and colleagues\textsuperscript{15}, renumbering only atrial tissue points could reduce memory and CPU usage to between 10 and 30%. Exclusive use of integer or binary input and output files would also enhance computational performance.

The correct approach is to implement a multi-scale model that captures key features of myocyte architecture and surface geometry at the lowest appropriate spatial resolution. The present work, as well as previous studies by our group\textsuperscript{5}, demonstrates that this is possible. Likewise, the reaction-diffusion equation can be solved accurately over much of the cardiac cycle with time steps substantially greater than the fixed 0.01 ms interval employed here, but more sophisticated adaptive solution schemes are required.

**Atrial fiber tracts and preferential electrical propagation**

Analysis of specific regions at 50×50×50 µm\textsuperscript{3} resolution enabled us to relate the spread of electrical activation to 3D myofiber arrangement reconstructed within the region, at the same resolution. There was little difference in the activation spread predicted with isotropic and anisotropic electrical properties for atrial regions where myofiber organization was heterogeneous. A typical example is presented in Figure S5 for a segment from the lower anterior LA wall (see Figure S4D-F) where myofiber orientation is highly disordered. This is contrasted with results presented in Figure S6 for a segment (2.5×3×5 mm\textsuperscript{3}) from the LIPV at its junction with the PLA, where atrial myofibers form an organized bundle. Here, as one would expect there are substantial differences in the electrical propagation predicted with isotropic and anisotropic electrical properties. In the isotropic case (the left panel of Figure S6A), activation spreads uniformly at ~0.7 m/s. However, preferential spread is evident in the right panel of Figure S6A, where CV is ~1.1 m/s in the myofiber direction and ~0.6 m/s transverse to this. In Figures S6B, activation isochrones for isotropic and anisotropic electrical properties, respectively, are rendered on tracts obtained within this segment using high resolution fiber tracking. In the latter case it is clear that the preferential directions of propagation through the segment are slightly curvilinear reflecting the variation of local myofiber tracts within it.

Atrial activation spread has been reconstructed at high spatial resolution in 3D on the endocardial surface using bipolar contact electrodes in normal dog\textsuperscript{16} and human\textsuperscript{17} hearts. High resolution epicardial surface measurements have also been obtained in a series of atrial regions in dogs\textsuperscript{18} and man\textsuperscript{19} again using contact electrodes, and in an isolated supported sheep heart preparation using...
optical mapping\textsuperscript{20-22}.

While measured local activation patterns correspond very closely with those presented in this paper, predicted activation times appear to be longer than those that have been reported. Total activation times in the human atria were 105 ± 9 ms, but were around 70 ms in the dog. The difference is likely due to the conductivities assigned in our model. For regions including the CT, PMs and BB, characterized by uniform myofiber alignment, axial CV was \textasciitilde 1.1 m/s along the fiber axis whereas CV was around \textasciitilde 0.67 m/s in the remaining isotropic regions. Conduction velocities estimated from epicardial surface measurements in working atrial myocardium\textsuperscript{20,23} range from 0.32 to 1.03 m/s. Estimates between 0.7 and 1.3 m/s have been reported for the CT\textsuperscript{24,25}, but optical mapping studies in the sheep\textsuperscript{21} suggest maximum velocities greater than this. Finally, measured velocities for the PMs\textsuperscript{26,18} range from 1.0–1.54 m/s and for BB\textsuperscript{26,27} from 0.92–1.67 m/s. For the specialized conduction pathways, therefore, CVs are all at the low end of measured values.

\textit{Effects of ectopic stimulation from PV sleeves}

The effects of myofiber anisotropy on electrical stability following ectopic stimulation from the PLA were investigated in a separate series of simulations. \( S_2 \) stimuli were applied at 8 different locations on the PV sleeves (2 different sites for each of the four PVs, see Figure 8A in main paper) following \( S_1 \) activation close to the SAN. All times were related to the start of the vulnerable period, defined as the time at which successful propagation was first achieved with \( S_2 \) stimulation.

In Figure S7, we present the results of a further simulation in which an \( S_2 \) stimulus was applied at a second site on the LSPV sleeve (location 2 in Figure 8) within the vulnerable period following \( S_1 \) activation near the SAN. This has been selected because, together with Figure 8, it provides a sense of the range of results obtained. Here, the difference between activation spread with anisotropic and isotropic electrical properties is least marked, but there are still qualitative differences in the rate and direction of initial activation spread. Activation reached the midline of the SPB \textasciitilde 16 ms later following \( S_2 \) stimulation in the anisotropic case than in the isotropic case. In the former, conduction from the veno-atrial junction into the SPB transverse to the fiber axis was not blocked, but propagation in this direction was very slow, reflecting the anisotropic electrical properties. In both isotropic and anisotropic cases, activation spread is slowed by the effects of the short coupling interval on CV restitution, as well as the current source-load mismatch that results from the abrupt tissue dilatation between PV sleeve and veno-atrial junction.
Supplemental Figures and Legends

Figure S1: Processing and segmentation of the atria images. (A) Typical composite 2D image section prior to processing. (B) A after application of Hessian filter and region growing correction. (C) B after dilation and erosion process to close boundaries. (D) Section at same location as A after 3D interpolation and smoothing.
Figure S2: Semi-transparent 3D reconstruction of atrial chambers. Color mapping selected to emphasize endocardial structures and variations in wall thickness. (A) Antero-superior view. (B) Inferior view showing tricuspid valve (TV) and mitral valve (MV) annuli.
Figure S3: Inclination angle $\alpha$ and transverse angle $\beta$ of local atrial myofiber vector. The red arrow indicates myofiber orientation at a point in a horizontal X-Y plane. The inclination angle $\alpha$ is the projection of this vector onto a vertical plane parallel to the epicardial boundary. This angle is measured with respect to the horizontal. The transverse angle $\beta$ is the projection of the fiber vector onto the horizontal plane, with respect to the surface tangent plane defined above.
**Figure S4:** Myofiber architecture in image segments from anterior left atrium (LA) wall. (A) Location of superior left atrial appendage (LAA) segment (5x15x2.5 mm, centered at Z = 18.3 mm - see Figure 1). (B) 3D reconstruction of image segment. (C) Inclination angle $\alpha$ and transverse angle $\beta$ as functions of wall thickness - 0 and 100% correspond to epicardial and endocardial surfaces, respectively. (D) Location of inferior LAA segment (8x7.5x2.5 mm, centered at Z = 7.8 mm - see Figure 1). (E) 3D reconstruction of image segment with epicardial surface outermost. (F) High resolution fiber tracking for subvolume in E).
Figure S5: Effects of uniformity of myocyte alignment on local spread of electrical activation in atrial myocardium in the absence of ordered myofiber organization assuming isotropic and anisotropic electrical properties, respectively. Segment from inferior LA in Figure S4E. Activation time color spectrum indicated.
Figure S6: Effects of isotropic and anisotropic electrical properties on activation spread modeled on a pulmonary vein (PV) segment (2.5×3×5 mm$^3$, 50×50×50 μm$^3$ resolution) from the junction of the lower left PV with the posterior left atrial wall. (A) Time-course of depolarization at 1 ms intervals. Red region indicates depolarized tissue. (B) Activation isochorones rendered on tracts reconstructed within segment by high resolution fiber tracking. For both isotropic and anisotropic cases, the segment has been rotated by 180° to provide a more complete view of activation spread with respect to myofiber architecture.
Figure S7: Comparison of effects of anisotropic and isotropic electrical properties on epicardial spread of ectopic electrical activation in the PLA induced with $S_1$-$S_2$ stimulation protocol. The $S_1$ stimulus is "normal" SAN pacing as shown in Figure 5. The time-course of activation in PLA following an $S_2$ stimulus delivered at location 2 during the vulnerable period. Potentials are color coded as displayed in Figure 8B.
Supplemental References


