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Key Words: electric anisotropy ■ electrophysiology ■ fibrosis ■ infarct border zone ■ myocardial architecture ■ normal activation ■ reentrant arrhythmia

Three-Dimensional Impulse Propagation in Myocardium: Arrhythmogenic Mechanisms at the Tissue Level

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Abstract: Impulse propagation in the heart depends on the excitability of individual cardiomyocytes, impulse transmission between adjacent myocytes, and the 3-dimensional arrangement of those cells. Here, we review the role of each of these factors in normal and aberrant cardiac electric activation, with particular emphasis on the effects of 3-dimensional myocyte architecture at the tissue scale. The analysis draws on findings from in vivo and in vitro experiments, as well as biophysically based computer models that have been used to integrate and interpret these experimental data. It indicates that discontinuous arrangement of myocytes and extracellular connective tissue at the tissue scale can give rise to current source-to-sink mismatch, spatiotemporal distribution of refractoriness, and rate-sensitive electric instability, which contribute to the initiation and maintenance of reentrant cardiac arrhythmia. This exacerbates the risk of rhythm disturbance associated with heart disease. We conclude that structure-based, multiscale computer models that incorporate accurate information about local cellular electric activity provide a powerful platform for investigating the basis of reentrant cardiac arrhythmia. However, it is important that these models capture key features of structure and related electric function at the tissue scale. (Circ Res. 2013;112:834-848.)

Key Words: electric anisotropy ■ electrophysiology ■ fibrosis ■ infarct border zone ■ myocardial architecture ■ normal activation ■ reentrant arrhythmia
for ≈100 years. However, understanding exactly how they occur and predicting the likelihood that they will do so have proved much more elusive.

Knowledge of 3D impulse propagation in the heart is dominated by views from quite different points on the continuum of spatial scales. This reflects our capacity to image and resolve 3D structure and to make related functional measurements. At the cell level, there has been intense focus on and resolve 3D structure and to make related functional measurements. At the cell level, there has been intense focus on the contributions of transmembrane ion channels and trans-sure (HF) increases the extent and heterogeneity of structural discontinuity.10,13 There is evidence that electric dysfunction and structural remodeling at cell and tissue levels can give rise to ectopic activation,14 and local propagation delays that support reentry.15 However, key aspects of impulse propagation at the tissue scale may not be captured fully by computer models that represent electric properties as continuous. Improved understanding of mechanisms at this level that are responsible for reentrant arrhythmia is important. Therefore, robust representation of impulse propagation at the tissue scale is necessary for multiscale modeling of cardiac rhythm disturbance.

In this article, we outline the biophysical basis for 3D impulse propagation in the myocardium, with particular emphasis on the effects of myocyte architecture and structural discontinuities at the tissue scale. We consider how structural features at this level determine normal impulse propagation in ventricles, atria, and the cardiac conduction system. Finally, we review areas in which quantitative analyses of 3D propagation at the tissue level are providing improved understanding of mechanisms that trigger and sustain arrhythmia.

Cell Activation

Cell activation is driven by the net inward transmembrane current, which gives rise to rapid depolarization. In cardiomyocytes, this current is carried by sodium channels ($I_{\text{Na}}$) and, to a lesser extent, by $\alpha$-type calcium channels ($I_{\text{Ca,L}}$), in which conductivities are both membrane potential–dependent and time-dependent.

This is illustrated in Figure 1, in which restitution relations for cellular activation have been simulated using a Luo Rudy dynamic (LRd) activation model. Here, a single S2 stimulus is applied soon after a series of S1 conditioning cycles, and this is repeated for a range of coupling intervals. Although repolarization is necessary to reset sodium channel activation and inactivation gates, there is a time delay between the completion of repolarization and full recovery of $I_{\text{Na}}$ in the subsequent activation. Furthermore, $I_{\text{Ca,L}}$ provides a significant fraction of the inward current flux during successful depolarization at short coupling intervals.

The dynamics of repolarization clearly have a significant impact on inward current when the time interval between successive activation is short, as is the case in reentrant arrhythmia. Repolarization is the result of outward current flow mainly carried by potassium channels, including the transient outward current ($I_{\text{to}}$), which contributes to early repolarization (not captured in the LRd model used in Figure 1), the
period. Also noteworthy is the fact that activation dynamics as coupling interval is reduced within the relative refractory period. CV in the cable decreases progressively as CI increases during the relative refractory period, but \( I_{\text{Na}} \) contributes to depolarization at short Cls. 

**Impulse Transmission Between Myocytes**

The first biophysical analyses of impulse propagation in cardiac tissue used 1-dimensional (1D) cable theory. However, even aligned tracts of cardiac tissue, such as Purkinje fibers, are 3D arrays of individual cells, and their electric properties cannot be represented fully by continuous 1D models. However, even aligned tracts of cardiac tissue, such as Purkinje fibers, are 3D arrays of individual cells, and their electric properties cannot be represented fully by continuous 1D models. Despite this, classic 1D cable theory provides a useful conceptual framework for considering impulse propagation in cardiac tissue and the role of current source-to-sink matching, in particular.

In Figure 1C, we present a snapshot of activation propagating in a continuous cylindrical tract with uniform LRd membrane kinetics. The distribution of transmembrane voltage ahead of the depolarization wavefront is the result of discharge of the depolarized cell membrane and redistribution of inward current. An increase in inward current flux heightens the rate of the depolarized cell membrane and redistribution of inward current. Atrial fibrillation occurs when membrane potential-dependent and time-dependent behaviors of \( I_{\text{Na}} \) also ensure that the dynamics of cellular activation can be modulated by external factors, as well as action potential (AP) morphology. For instance, hyperkalemia causes partial depolarization of the resting myocyte membrane, resulting in closure of some sodium channel inactivation gates and reduced \( I_{\text{Na}} \) on activation.

This provides a conceptual framework for linking the cellular activation restitution in Figures 1A and 1B with CV restitution in a continuous tract of cells. CV in the cable decreases as coupling interval is reduced within the relative refractory period. Also noteworthy is the fact that activation dynamics for single cell and cable differ; in the latter, the coupling interval for successful propagation increased.

The current that flows directly between cardiomyocytes during impulse transmission and the small molecules required for cell signaling pass through gap junctions or connexins located mainly in arrays within the intercalated disks (ICDs). The ICDs are arranged transverse to the myocyte axis and, in addition to electric connection, provide physical coupling between myocytes. Gap junction channels consist of a pair of aligned connexins (hemichannels) each located in the apposed cell membrane and consisting of 6 connexin molecules. Some individual connexins are also observed along the lateral cell membrane. The main cardiac connexins are Cx40, Cx43, and Cx45. Of these, Cx40 is found in the atrium and in the ventricular conduction system, Cx43 is the predominant connexin in working ventricular myocardium but is also colocated with Cx40 in the atria, and Cx45 is expressed with Cx40 in the bundle branches. Connexins are voltage-sensitive; both Cx43 and Cx40 inactivate partially with altered junction voltage (time constant, \( \approx 150 \text{ ms} \)). Gap junction conductivity is also modulated by intracellular Ca\(^{2+}\), pH, and phosphorylation.

The influence of gap junctions on impulse transmission has been investigated using computer modeling and closely related patterned cell culture experiments. In the former, propagation in discontinuous 1D strands of myocytes, each coupled via discrete resistive pathways, differed from the predictions of continuous cable theory. Conduction within cells was rapid, with relatively long propagation delays across the junctions between them, and these delays increased as gap junction coupling was reduced. CV decreased progressively over several orders of magnitude as gap junction resistance was increased, and \( I_{\text{Ca,L}} \) played an increasingly important role in this slow propagation. Shaw and Rudy concluded that the “increase of safety factor during reduced coupling suggests a major involvement of uncoupling in stable slow conduction in infarcted myocardium, making microreentry possible.” Kléber...
et al. tested these predictions in a sequence of elegant cell culture experiments. They observed saltatory conduction in single strands of myocytes but noted that lateral averaging smoothed propagation in multicellular strands. Increasingly, slow conduction occurred with progressive gap junction uncoupling; meandering and even retrograde propagation were seen in thick myocyte strands with complete uncoupling.

It is argued that membrane potential during early repolarization is critical to impulse propagation between adjacent cells, particularly when coupling resistance between them is high, and that the downregulation of \( I_{\text{TO}} \), which occurs in HF and atrial fibrillation (AF), may contribute to stable slow propagation under these circumstances. Studies in pairs of isolated cardiomyocytes electrically coupled via high resistance demonstrated that propagation delays were reduced with inhibition of \( I_{\text{TO}} \). This reflects the fact that \( I_{\text{TO}} \) is responsible for early repolarization and sets the membrane potential that charges the adjacent cell membrane.

Total Cx43 in the ventricular myocardium is reduced in most forms of structural heart disease, and its distribution becomes more heterogeneous. This has led to the view that the increased probability of arrhythmia in this setting is a direct result of delayed impulse transmission between myocytes. However, studies of Cx43 knockout mice suggest that the relationship between Cx43 expression and CV may be more complex than this. A variety of approaches has been used to develop experimental models that mitigate early developmental effects of Cx43 deficiency. These include a heterozygous Cx43-deficient strain in which Cx43 is reduced to \( \pm 50\% \); a conditional knockout in which Cx43 expression decreases progressively with age, and a mutant strain in which Cx43 can be deleted at any time point by application of 4-hydroxytamoxifen. No significant differences in CV or CV restitution were found with Cx43 deletion up to \( \pm 60\% \). However, depletion of Cx43 by \( \pm 80\% \) was associated with CV reduction, increased CV anisotropy, and increased risk of ventricular tachycardia (VT) and sudden cardiac death.

These results suggest that impulse propagation between cells in the ventricular myocardium is relatively insensitive initially to reduced Cx43 expression. This is different from the findings outlined for patterned cell culture preparations and likely reflects the fact that distributions of sodium channels, gap junctions, and cell dimensions in cultured neonatal rat ventricular myocytes differ considerably from the intact ventricular myocardium. Reductions of total Cx43 in ventricular myocardium \( \leq 50\% \) have been reported for end-stage HF in humans and in animal HF models. It has been argued that these changes would not decrease CV sufficiently to support reentry. The counterview is that cardiomyopathy is also associated with heterogeneous spatial distributions of Cx43. Under these circumstances, some regions in some diseased hearts will reach the threshold of Cx43 depletion at which impaired impulse propagation provides a substrate for reentry.

The only serious alternative to the generally accepted understanding of impulse transmission between cells is the electric field mechanism (ephaptic transmission) first proposed by Sperelakis et al. These researchers argued that activation spreads along tracts of cardiac cells in a saltatory fashion driven by the negative potential that develops in the restricted cleft space between cells when an AP develops in the prejunctional membrane. The fact that transmembrane sodium channels are concentrated around the ICDs has been seen as providing a mechanistic framework for ephaptic transmission. Computer models of 1D myocyte strands in which sodium channels were colocated with gap junctions at the interface between adjacent cells suggest that ephaptic coupling increases CV at low gap junction conductivity and vice versa. One interpretation of these findings is that both mechanisms act in concert to stabilize impulse transmission between myocytes. This provides a basis for the observation that CV is relatively insensitive initially to reduced Cx43 coupling.

Cardiac fibroblasts, which are responsible for development and regulation of the cardiac extracellular matrix, are the most prominent cell type in the heart and are arranged in a network that surrounds cardiomyocytes. Myocardial injury leads to the formation of activated fibroblasts or myofibroblasts, which are not present in the normal myocardium and exhibit significantly greater rates of proliferation and collagen production than fibroblasts. Fibroblasts and myofibroblasts may influence cardiac electric function via paracrine interactions with cardiomyocytes or mechanoelectric feedback. However, the possibility that they affect electric activity and modulate impulse transmission by direct electrotonic interaction has attracted considerable attention recently. This has been driven by demonstrations of bidirectional coupling among myocytes, fibroblasts, and myofibroblasts via functional gap junctions.

A range of membrane ion channels, transporters, and pumps has been identified in cardiac fibroblasts and myofibroblasts. In particular, membrane ion channels that carry inwardly rectifying K+ currents and various voltage-dependent outward K+ currents have been characterized in adult rat ventricular fibroblasts and myofibroblasts. Measured resting membrane potentials for fibroblasts and myofibroblasts are significantly less negative than associated cardiomyocytes. Computer models based on these electrophysiological data have been used to investigate potential effects of electrotonic coupling between cardiomyocytes and fibroblasts. More recently, this approach has been extended to include interactions among myofibroblasts and cardiomyocytes. These analyses showed that the fibroblast/myofibroblast network could act as a current source for adjacent inactive cardiomyocytes and as a sink during activation because of membrane potential differences between them. Partial depolarization of the myocyte membrane at rest, reduced \( I_{\text{Na}} \), altered AP morphology, and decreased APD were predicted. The degree of modulation depended on fibroblast electrophysiology and the physical nature and extent of coupling between the fibroblast/myofibroblast network and cardiomyocytes. Experimental data in each of these areas are limited, and this issue needs to be addressed.

Experimental studies performed in cocultures of cardiomyocytes and cardiac fibroblasts provide further insights. In this setting, fibroblasts undergo a phenotype shift to smooth muscle actin–positive myofibroblasts that exhibit gap junction coupling among themselves and with cardiomyocytes. Myofibroblast density–dependent reductions in cardiomyocyte resting membrane potential, CV slowing (\( \leq 35\% \)), and successful propagation across fibroblast barriers \( \leq 300 \mu m \)
wide⁴ were observed in patterned neonatal rat ventricular myocytes coated with cardiac myofibroblasts. Results from these coculture studies should be treated with some circumspection; because there are structural and functional differences between neonatal rat myocytes and adult cardiomyocytes,²⁸ myofibroblast electrophysiology was not characterized, and gap junction coupling differs from that observed in adult ventricular myofibroblast myocyte cocultures.³⁹

Despite these qualifications, it is evident that direct electrotonic coupling between the fibroblast/myofibroblast network and cardiomyocytes could contribute to electric dysfunction in heart disease. Finally, differentiation of human atrial myocytes into myofibroblasts has been linked with the expression of voltage-gated sodium channels,⁴³ suggesting that myofibroblasts, unlike fibroblasts, also could exhibit intrinsic activity.

Myofiber Architecture and Impulse Propagation

The 3D arrangement of the ICDs that couple adjacent myocytes is a key determinant of electric anisotropy in myocardial tissue. ICDs are oriented transverse to the axis of the contractile lattice and are distributed irregularly across the ends of myocytes and along the lateral margin (Figure 2A). In a working ventricular myocardium, each cell is connected to ≈11 neighboring cells, although the coupling ratio is less for specialized conduction tracts, such as the crista terminalis.⁴⁴ Locally, myocytes have a near-parallel alignment, and this orientation is defined as the myofiber direction (Figure 2B). Electrical activation propagates preferentially along the cell axis, whereas the rate at which activation spreads transverse to this reflects the extent of lateral coupling between adjacent myocytes. Historically, it was assumed that lateral electric coupling is spatially uniform at any point within the myocardium. That is, the electric properties of myocardial tissue were viewed as isotropic transverse to the myofiber direction.

Systematic 3D investigation has revealed a further level of structural anisotropy in the ventricular myocardium.⁵⁰,⁵⁵ Ventricular myocytes are arranged in layers 4 to 6 cells thick that are separated by clefts of perimysial connective tissue, across which there is little direct cell-to-cell coupling (Figure 2C). These layers form a branching network, and they have been referred to as sheets,⁴⁵ myolaminae,⁴⁶ and, more recently, sheetlets.⁶ The laminar architecture described here has been confirmed with contrast-enhanced high-resolution magnetic resonance imaging⁶ and diffusion tensor magnetic resonance imaging.⁵ Therefore, ventricular tissue is not uniformly connected transverse to the myofiber direction. Instead, it is an orthotropic material, with 3 distinct structural axes defined at any point by the myofiber direction and myolaminar orientation. Transmural myofiber rotation adds additional complexity to this cellular organization. Viewed from the outside, fiber orientation is approximately –60° with respect to the circumferential direction at the epicardial surface and rotates counterclockwise through the wall to approximately +90° in the subendocardial region.

Patterned cardiomyocyte monolayers can capture many features of the 2D cellular architecture illustrated in Figure 2B, in that cells are polarized and tightly coupled electrically.²¹ Furthermore, important insights have been gained in this setting regarding the role of source-to-sink current matching in impulse propagation.

Conduction slowing and unidirectional block have been demonstrated at abrupt tissue expansions in patterned cell culture²¹,⁴⁶ (Figure 3A and 3B) and in ventricular muscle slices.⁴⁷ This is caused by source-to-sink current mismatch, the result of current flux from the isthmus into the much larger distal region, and a subsequent increase in wavefront curvature within that region⁴⁶ (Figure 3C). Distinct phases also develop in the AP at the entrance to the expansion,²¹ and computer simulations suggest that slow initial depolarization inactivates sodium channels and ³Na⁺ is reduced, whereas ³Ca,L is augmented.⁴⁸ Of particular interest is the rate dependence of conduction slowing⁴⁶ and block,⁴⁶ which shifted to lower frequencies as the relative width of the expansion increased⁴⁶ (Figure 3D). This occurs at a much lower frequency than would be expected based on isolated cell repolarization kinetics.

Matched in vitro and in silico experiments⁴⁹,⁵⁰ have been used to investigate how repetitive tissue branching may contribute to slow stable conduction. Propagation along a tract of cultured cells with periodic blind-ended branches produced slow conduction with a high safety factor through 2 mechanisms. Branches acted as current sinks for approaching activation waves, initially slowing propagation but, once excited,
provided depolarizing current that supported downstream activation.

Because it is difficult to map intramural activation in 3D at high spatial resolution, structure-based computer models have been used to simulate impulse propagation inside the ventricular wall. Models of the intact ventricles have been used to investigate the influence of myofiber orientation and structural heterogeneities, such as blood vessels and endocardial trabeculation, on activation spread. However, because most treat ventricular myocardium as a continuum, they provide limited insight into the effects of discontinuity on 3D impulse propagation at the tissue level.

The effects of structural discontinuity at the tissue scale have been investigated in a structurally detailed, image-based representation of myocyte and collagen architecture (1.56-µm³ voxel dimensions) throughout a transmural segment from the rat LV-free wall. Cleavage planes between muscle layers were explicitly incorporated into the model. Electrical coupling between myocytes transverse to the local myofiber direction was considered to be uniform within layers, but there was no direct electric coupling between myocytes across cleavage planes. The predicted spread of electric activation from an intramural point stimulus in the LV was most rapid along the myocyte axis and somewhat slower transverse to the cell axis in muscle layers, but much slower again in the direction perpendicular to the muscle layers; transverse conduction velocities divided in a ratio of ≈2:1. This reflects the discontinuities introduced by cleavage planes and the tortuous propagation pathways normal to muscle layers. A limitation of this model was the fact that activation was simulated on a relatively small transmural segment rather than the intact 3D heart.

Model predictions that the electric properties of ventricular myocardium are not transversely isotropic with respect to the myofiber direction have been tested in 2 pig heart studies that combined high-resolution intramural mapping, detailed structural measurement, and structure-based modeling. Bulk conductivity transverse to the fiber axis divided in a ratio of ≈2:1, with maximum conductivity in the direction of myolaminae and minimum conductivity perpendicular to this. Caldwell et al used a high-resolution intramural electrode array to reconstruct the 3D spread of electric activation from point stimuli within the array. These data were then related to myofiber direction and laminar orientation throughout the tissue volume bounded by the recording array. Consistent with model predictions, measured conduction velocities separated in the ratio ≈4:2:1. The maximum velocity was in the myofiber direction. Transverse to this, the intermediate velocity was aligned with myolaminae, whereas the minimum velocity was perpendicular to laminar orientation (Figure 4).

The functional consequences of these discontinuous, inherently orthotropic, electric properties warrant further comment. Laminar myocyte architecture increases propagation safety, because passive current load is constrained locally. In addition, one would expect relatively rapid transmural activation in sinus rhythm, when the endocardial surface of the ventricles is excited via the Purkinje network, because myolaminae have a predominantly radial orientation. However, much slower propagation will occur normal to muscle layers after an intramural ectopic stimulus.

Anatomically based computer models provide a framework for predicting 3D impulse propagation in the atria. This has motivated the development of atrial models of increasing complexity that have incorporated realistic representations of 3D chamber geometry and regionally varying electrophysiological properties. However, few have included detailed information about atrial myofiber orientation. Most have assumed that atrial electric properties are isotropic, introduced prescribed local anisotropy to account for the role of myocyte tracts such as the Bachman bundle, or used rule-based methods that seek to capture key features of qualitative descriptions of atrial myofiber anatomy. In contrast, more recent anatomic models incorporate comprehensive representations of myofiber architecture, as well as atrial geometry. Myofiber tracts throughout the atria have been reconstructed using structure-tensor analysis of image volumes acquired with serial surface imaging and contrast-enhanced computed tomography from sheep and dog atria, respectively. These data match previous, more qualitative, descriptions of muscular architecture in the human atria obtained using dissection, macrophotography, and visual tracing of fiber tracts.

The ordered myofiber architecture that characterizes the ventricles is not replicated in the atria. Although muscle...
bundles in which myocytes are aligned over relatively long path lengths can be identified throughout the atria, myofiber orientation changes abruptly across much smaller spatial scales in many regions. Furthermore, laminar myocyte arrangement and smooth transmural variation in myofiber orientation are much less evident in the atria than the ventricles.

Simulations of electric activation on detailed atrial anatomy demonstrate that the ordered alignment of myocytes in some atrial regions plays an important role in normal atrial activation spread (Figure 5). They also confirm that specialized myofiber tracts, such as Crista Terminalis (CT), pectinate muscles (PM), and Bachman bundle, have greater anisotropy ratios and CVs than a working atrial myocardium. Finally, the complex myofiber architecture of the posterior left atrium (PLA) seen in Figure 5B gives rise to marked activation time dispersion across this region (Figure 5C).

The cardiac conduction system is responsible for initiation and coordinated distribution of electric activation throughout the heart. The cardiac conduction system consists of 3 main components, the sinus node, the atrioventricular node (AVN),
and the His-Purkinje system (HPS). Anatomic descriptions of the cardiac conduction system have been available for some time, but there have been few attempts to quantify the 3D geometry of the system or to characterize its components systematically at the cellular level.

Detailed structural data have been acquired for sinus node and AVN, and key features (extracellular connective tissue matrix, cell types, gap junctions, and others) have been identified and reconstructed in 3D at high spatial resolution in both. Serial sections from throughout these structures were alternately processed with histological stains or labeled immunohistochemically, then imaged and incorporated into accurately registered 3D anatomic models. Two distinct cell types were observed in the sinus node. A network of small cells that did not express Cx43 was seen in the central pacemaker region, whereas sinus node cells in the periphery were large, aligned in parallel, and often expressed Cx43. In the AVN, atrial myocardium, inferior nodal extension, penetrating bundle, His bundle, and ventricular myocardium were characterized based on Cx43 and neurofilament expression, as well as connective tissue staining. These data were incorporated into a comprehensive 3D representation of the cellular architecture of the AVN (Figures 6A–6C). In the next section, we describe how this model has been used to investigate the structural and functional bases of reentry in the AVN.

Recently, high-resolution, contrast-enhanced computed tomography has been used to reconstruct the 3D arrangement of the conduction network within a mammalian heart for the first time. This will provide a means for investigating how the topology of the network affects conduction safety and facilitates development of an integrated anatomic model of the cardiac conduction system.

**Triggers for Reentrant Arrhythmia: A Role for Tissue Architecture?**

The cellular origins of the early after depolarizations (EADs) and delayed after depolarizations (DADs) that trigger premature activation are now well-understood. EADs are caused by reactivation of I_{Ca,L} as a result of APD prolongation. However, both EADs and DADs can be generated by waves of intracellular calcium release that occur as a result of impaired intracellular calcium handling.

Much less clear is how unstable cycles of intracellular calcium release are synchronized to provide sufficient current to trigger electric propagation in the surrounding myocardium. Xie et al used computer modeling to estimate the number of normal myocytes that must be brought to threshold to trigger a propagated AP. For a 1D strand of myocytes, the numbers required were 70 and 80 for an EAD and a DAD, respectively. In 2D tissue, these numbers increased to 6940 and 7854, and in 3D myocardium the numbers were 696910 and 817280. This analysis assumes uniform coupling between myocytes and, for this reason, the 3D estimate is almost certainly overstated. The ordered arrangement of cleavage planes in ventricular myocardium constrains activation spread locally to 2D and, therefore, increases propagation safety. Decreased coupling between myocytes, for instance, because of reduced gap junction expression or fibrosis, would further reduce these figures. It is evident that significant synchronization of EADs and DADs is necessary to overcome the “robust protective effects of source-to-sink mismatch.” For the reasons outlined, the probability that unstable cellular activity will successfully drive premature electric activation is greatest in quasi-1D structures, such as the HPS, or in the PV sleeves, where there is marked spatial variation in the electric properties of cells, the 3D arrangement of myocytes, and electric coupling between them.

The intracellular instability that gives rise to calcium waves is manifested at cell and tissue levels as repolarization alternans, which is the result of interaction between membrane repolarization kinetics and intracellular calcium handling. The alternans rhythm that occurs in heart disease almost certainly reflects impaired calcium cycling. However, APD alternans is influenced by APD restitution properties (the relationship between APD and diastolic interval). APD restitution slope increases with heart rate and, if sufficiently steep, might also drive APD alternans. Spatially discordant alternans, in which electric rhythm in adjacent heart regions is out of phase, can be caused by nonuniform activation delays attributed to the presence of structural barriers. Discordant alternans has been simulated in a 2D domain with normal cell activation properties that also contained nonuniform discontinuities resembling patchy fibrosis. As stimulus rate increased, progressively more discordant alternans rhythm was centered on the fibrosis. This was characterized by marked activation...
time dispersion and extreme local variation in APD alternans within the fibrotic region. This instability gave rise to slow conduction, regional block, and reentry. In this simulation, rate-dependent instability was the trigger for reentry but also contributed to the dynamic substrate that sustained it.

Evidence that cardiac structural heterogeneities may contribute to EAD formation has been provided with the patterned cell culture preparation already shown in Figure 3. This consists of 2 wide cell monolayers connected by a thin isthmus and, at high stimulus rates, propagation into the distal expansion was characterized by rate-dependent conduction block. However, at low stimulus rates, AP prolongation at the entry to the distal expansion gave rise to EADs that propagated retrogradely along the isthmus (reflection). In some cases, reactivation at the distal expansion set up a rotor that seeded further retrograde activation waves along the isthmus. The primary mechanism underlying these findings is the asymmetrical source-to-sink loading at the interface between isthmus and expansion, and this raises the possibility that a microreentrant circuit could be formed through the apposition of appropriate heterogeneities. In that case, rate-dependent properties associated with heterogeneous structure might enable the region to trigger ectopic activity but also act as a substrate for reentry.

AF: Influence of Tissue Structure and Structural Remodeling

AF is the most common heart rhythm disturbance, and its incidence increases with aging and heart disease. It gives rise to a cascade of structural and functional remodeling that sustains reentry. Derangement of intracellular calcium handling increases the probability that, after depolarizations, will trigger ectopic activity. In AF, \( I_{\text{Ca,L}} \) and \( I_T \) decrease, whereas inwardly rectifying current and other transmembrane potassium currents increase. In general, the net effect of these changes is to reduce APD, but reduced \( I_T \) may prolong late atrial repolarization in congestive HF, increasing the probability that DADs will occur with \( \beta \)-adrenergic stimulation. CV is thought to be reduced in AF as a result of decreased gap junction expression, although the extent of these changes remains unclear. Furthermore, aging, congestive HF, and AF are associated with marked interstitial fibrosis that produces conduction abnormalities. The connective tissue that separates myofiber bundles thickens increasing electric anisotropy, whereas replacement fibrosis may interrupt longitudinal propagation in some regions. Finally, congestive HF gives rise to atrial dilation. All of these factors increase the probability of reentry in the atria. We review experimental and modeling studies that have addressed the origins and role of altered impulse propagation in this setting.

The critical role that the PVs play in triggering paroxysmal AF is clearly established by the success of PV isolation in reversing this condition. However, the mechanisms responsible for the initiation of wavebreak in the PLA are still not fully understood. Hypotheses include source-to-sink mismatch caused by abrupt changes in wall thickness and myofiber orientation at the venoatrial junctions and spatial variation in repolarization between the PV sleeves and adjacent atrial myocardium in the PLA.

The effects of PLA structure on vagally mediated AF have been investigated in isolated Langendorff-supported sheep hearts. Optical mapping revealed preferential propagation between the left and right PVs (along the septopulmonary bundle) in sinus rhythm. With burst stimulation from left inferior and right superior PVs, waves propagating into the PLA experienced delay and triggered reentry by breaking at boundaries along the septopulmonary bundle where there were abrupt changes in wall thickness and fiber direction. It was argued that sink-to-source mismatch in these regions resulted in low safety for propagation. Zhao et al recently completed comparable in silico studies using the image-based model of sheep atrial anatomy described. The model replicated the nonuniform activation time dispersion observed experimentally in the PLA. It also showed that burst activation originating in the PV sleeves can trigger fibrillatory behavior that is sustained by reentrant activation around the venoatrial junctions, again consistent with experimental results. Using the model, they were able to demonstrate that abrupt changes in wall thickness and myofiber orientation delayed activation waves propagating into the PLA. However, sustained reentry occurred only if these factors were combined with nonuniform electrophysiological properties in the PLA and shorter APD in the PV sleeves than in the surrounding atrial myocardium.

The substrate for persistent AF is distributed across a wider range of atrial regions than is the case for paroxysmal AF. Narayan et al identified rotors in the right atrium and left atrium (1:3, 2:1 respectively) in patients with chronic AF and reported that a single ablation at the focus of the rotor was sufficient to reverse AF in most cases. Recent experiments in a sheep model of persistent AF showed that rotors are more likely to form with acute atrial distension and that they stabilize at the edges of PMs in left and right atrial appendages. Again, this behavior was related to source-to-sink electric mismatch in these regions. However, simulations predicted that electric remodeling and heterogeneous distribution of stretch-activated channel conductances also contributed to filament stabilization. Berenfeld et al reported rate-dependent propagation delays and wavebreak adjacent to the junction between CT and PMs in an isolated sheep RA preparation. A structurally detailed computer model of the pig right atrial appendage was also used to investigate the breakdown of periodic electric activity in this region. A train of stimuli with reducing cycle length was applied at the CT junction, and rate-dependent unidirectional block and reentry were seen. These results were not affected by dispersion of repolarization. Although APD is longer in the CT than the PMs at resting stimulus rates, this difference reduces with increased rate. The model captured absolute APD and APD restitution accurately, and repolarization times were relatively uniform across PMs and CT for the stimulus rate at which reentry occurred. Model (and experimental) findings are, therefore, explained by the source-to-load mismatch caused by abrupt changes in tissue volume and myofiber orientation between PMs and CT and are exacerbated by the highly anisotropic electric properties of both.

There is a strong association between atrial fibrosis and risk of AF, and this has been confirmed in 2 recent studies. Burstein et al demonstrated altered Cx40/Cx43 expression, extensive
fibrosis, conduction abnormalities, and reduced thresholds for AF induction in dogs with pacing-induced congestive HF. After 4 weeks of recovery, connexin remodeling was reversed, but fibrosis, conduction disturbances, and AF promotion were not. More recently, it was shown that downregulation of a calcium-dependent signaling pathway that regulates fibroblast proliferation reduced fibrosis and prevented AF substrate development in a dog model of AF.73 Fibroblast/myofibroblast proliferation may contribute directly to electric instability in this setting. Computer modeling suggests that electronic coupling between fibroblasts and myocytes decreases APD and CV41 and, in a recent 2D simulation in which fibroblasts were distributed heterogeneously throughout a sheet of atrial myocytes, spiral waves stabilized in fibrotic regions, giving rise to electrograms that resemble the complex fractionated atrial electrograms seen in persistent AF.74 However, similar computer models that incorporate only the physical barriers to propagation associated with patchy fibrosis75,86 also exhibit tortuous conduction, activation delays, fractionated activation wavefronts, and rate-dependent instability. Prevention of fibroblast proliferation and differentiation, therefore, presents a potentially important new target for AF therapy.73

Atrial repolarization alternans appears to be a robust predictor of AF risk.75 APD alternans precedes episodes of AF and occurs at lower rates and with higher magnitudes in persistent than in paroxysmal AF.75 Atrial APD alternans is thought to reveal dynamic substrates for AF, and it has been linked with altered ion channel expression that occurs in AF76 and with altered intracellular calcium handling.75 However, it is likely that fibrosis amplifies regional rhythm instability because of its effects on local propagation and electrotonic current flow.64–66 Consistent with this, it has been shown in experimental and modeling studies that the spatial distribution of fibrosis in the PLA of sheep with pacing-induced HF markedly influences AF dynamics, with patches of fibrosis of varying sizes having the greatest impact.77

Cardiac Conduction System as a Substrate for Reentry

The HPS is thought to trigger a range of different ventricular arrhythmias in patients with structurally normal hearts.78 These include catecholaminergic polymorphic VT, which is strongly associated with increased Ca²⁺ release from the sarcoplasmic reticulum as a result of enhanced ryanodine receptor activity.70,72 The biophysics of cardiac impulse propagation suggests that the success of triggered activity will be greatest in quasi-1D structures, and cardiac Purkinje cells are more vulnerable to successful after depolarization formation than ventricular myocytes. It is argued that Purkinje cells are susceptible to EAD formation because of their higher membrane resistance and longer APD94 and to DAD formation as a result of higher sarcoplasmic reticulum content and the presence of membrane currents that reduce excitation threshold and promote triggered activity.82

Berenfeld and Jalife83 used one of the first anatomically realistic representations of the cardiac conduction system within a 3D ventricular model to demonstrate that induction of microcristoy within the HPS can give rise to sustained ventricular tachyarrhythmia. Furthermore, they showed that whereas the Purkinje network contributed to the initiation of arrhythmia, it was not necessary for its maintenance.

The effects of dispersion of repolarization on initiation of reentry in the HPS were investigated recently by Deo et al84 using an anatomicability realistic 3D model of the rabbit ventricles and conduction system. APD was longer in proximal than in distal Purkinje fibers, attributable in part to peripheral electronic coupling with ventricular myocytes. An S1 stimulus from the His bundle followed by an appropriately timed premature S2 stimulus in the distal Purkinje network gave rise to slow retrograde propagation that blocked proximally, because tissue in this region was refractory. This led to wavebreak with asynchronous activation spread via ventricular myocardium and reentrant activation through other components of the HPS. Conduction block and reentry could not be replicated with ectopic activation from proximal Purkinje fibers. They also showed that bundle branch reentrant tachycardia could be initiated by ectopic activation in the presence of slow ventricular conduction and conduction block in a bundle branch reproducing the findings of a similar 3D model.85

Heterogeneous repolarization also provides the substrate for reentrant AVN tachycardia.86 An analysis using the detailed 3D reconstruction of the AVN described (Figure 6) provides an example of what can be achieved when image-based computer modeling is combined with closely related experimental investigation.12 Electrical activation was simulated in the AVN using biophysically based and empirical activation models for the different tissue components. Two separate electric pathways were identified (one was slow conducting, through the inferior nodal extension; the second was fast conducting, through transitional tissue), and these were confirmed with optical mapping results acquired previously in the same specimen. It was then shown that the different electric properties of these pathways and inherent differences in their refractoriness provide a potential substrate for reentry (Figure 6D). An S1 stimulus from the His bundle produced retrograde propagation through both pathways. With an appropriately timed premature S2 stimulus from the same site, activation blocked along the fast conducting pathway because the transitional tissue had not repolarized but propagated through the slow pathway. This led to reentrant anterograde activation of the nodal tissue via the fast pathway (no longer refractory). This slow–fast reentry is consistent with the results of related experimental studies.87

Infarct Border Zone: Structural Remodeling and Reentry

The border zone (BZ) surrounding a healing or healed myocardial infarction can provide a substrate for reentrant VT. In the dog, myocytes are preserved after coronary occlusion in an epicardial layer above the infarct, and cellular and molecular remodeling in the canine epicardial BZ have been studied in detail. The initial response to myocardial infarction is myocyte necrosis, myofibroblast proliferation, cytokine release, inflammation, and edema, whereas scar formation and tissue remodeling occur over a period of days to weeks.84 Five days after coronary occlusion, side-to-side electric coupling between myocytes was reduced in the epicardial BZ,88 although this was not uniform.89 In the healed infarct, 3 to 10 weeks
after infarction, myocytes in the epicardial BZ were connected to 6.5±1.3 adjacent myocytes via ICDs compared with 11.2±1.0 in normal myocardium.90 End-to-end cell coupling was reduced by 22%, but side-to-side connections declined by 75%, the result of loss of ICDs along the cell margin.90 Electrophysiological changes in BZ myocytes in the subacute phase of infarct development include substantial reductions in resting membrane potential at 1 and 5 days,91 in peak phase of infarct development include substantial reductions in membrane potential and AP upstroke velocity returned to normal at 14 days,91 although peak \( I_{\text{Ca,L}} \) remained decreased36; AP morphology was restored by 2 months.91

Activation in the BZ of a chronic healed infarct is characterized clinically by long stimulus, site-dependent activation delays, and fractionated low-amplitude extracellular electrograms. Reasons advanced for this include decreased impulse transmission between cells because of reduced and heterogeneous Cx43 expression,3 cellular remodeling,93 and modulation of cell electrodynamics by myofibroblasts.17 A purely structural mechanism also has been proposed, including tortuous or zigzag conduction caused by infiltration of replacement and interstitial collagen in the BZ and the increased anisotropy of surviving myofiber tracts in this area.94

Vigmond et al95 used high-resolution magnetic resonance scans of a dog heart acquired 4 weeks after coronary artery occlusion to develop a 3D model of the ventricles. Infarct and epicardial BZ were identified, and myofiber orientation was characterized throughout. Electrical activation was simulated on this structure with modified LRd membrane kinetics. Transverse electric coupling was reduced uniformly throughout the BZ, and cell membrane kinetics were remodeled in the BZ to match data reported for dogs 5 to 14 days after coronary occlusion.88,92,93 Reentrant arrhythmia provoked using a standard clinical protocol resembled epicardial activation patterns mapped experimentally in the same animal after comparable VT induction. A closely related modeling study of the chronically infarcted rabbit42 demonstrated that myofibroblast infiltration in the BZ could increase the risk of arrhythmia. This analysis demonstrates that a uniform decrease in transverse conductivity combined with axial conduction slowing across the epicardial BZ can give rise to reentry. However, because these properties are homogenized in this model, we gain little insight into the possible roles of structural remodeling and tortuous propagation in the infarct BZ. To address this issue, Rutherford et al15 reconstructed myocyte and collagen organization at high spatial resolution (1-µm3 voxel dimensions) throughout LV tissue segments from rats 14 days after coronary occlusion. These transmural specimens contained infarct scar, BZ, and surrounding normal myocardium. Sparsely connected networks of myocytes penetrated the infarct BZ, and most terminated within it. However, a few strands as fine as 1 cell thick in places passed through the BZ transmurally, connecting adjacent normal myocardium to surviving subendocardial and subepicardial cell layers. At the interface between normal myocardium and BZ, lateral coupling between myocytes decreased abruptly over 200 to 300 µm.

Electrical activation was simulated on the network of connected myocytes throughout the image volume using a monomodular approach and using LRd membrane kinetics. It was assumed that cell electric properties and electric coupling between connected cells were uniform throughout. Stimulus site-dependent unidirectional propagation, rate-dependent activation delays, and conduction block were observed (Figure 7). These conditions provide a substrate for electric reentry and were the result of altered myocyte architecture alone. A component of the activation delay was caused by tortuous conduction across the BZ via surviving tracts of myocytes (zigzag conduction).94 However, the principal delays within the network were local rather than uniform and were the result of regional source-to-sink mismatch caused by abrupt changes in tract dimensions. Of particular interest, activation delays and conduction block in these regions were stimulus rate-dependent, again consistent with the mechanism of source-to-sink mismatch.

This analysis demonstrates that structural remodeling alone in the infarct BZ provides a sufficient substrate for reentry. It does not negate the potential influence of cellular and molecular remodeling or myocyte/myofibroblast interactions on impulse propagation within the BZ. We note that the structural remodeling seen in the BZ is entirely consistent with reported changes in gap junction distribution.90 Muscle layers are reduced to quasi-1D tracts; that is, lateral coupling is decreased and axial electric anisotropy is increased. Second, electrotonic coupling via myofibroblasts between adjacent closed tracts of myocytes could contribute further delay to propagation in the BZ. Finally, the existence of quasi-1D strands within the BZ increases the probability of successful propagation of afterdepolarizations, and it is possible that electromechanical coupling could contribute to this process.

**Fibrosis HF and VT**

HF is associated with increased risk of reentrant arrhythmia and sudden cardiac death,66 which reflects the extensive cellular and structural remodeling that accompanies this condition.2 Inwardly rectifying current, \( I_{\text{K,IR}} \), and delayed rectifier currents are reduced. Changes in sodium channel gating lead to sustained late sodium current and some reduction in peak \( I_{\text{Na}} \).2 At the whole-cell level, inward current may be affected by disruption of the transverse tubular system.97 The function of many components of the intracellular calcium handling system is also perturbed, with increased sodium–calcium exchange and reduced calcium storage in the sarcoplasmic reticulum.14 This electric remodeling extends APD and increases the likelihood that afterdepolarizations will occur. Furthermore, regional variability of cellular remodeling in HF gives rise to non-uniform repolarization, and this may exacerbate by altered extracellular potassium levels in the presence of ischemia.98 Gap junction expression and phosphorylation are reduced,1,29 which is thought to decrease CV, and structural remodeling also contributes to the conduction deficit. Interstitial fibrosis increases with age and HF progression.99,100 Perimysial components between adjacent muscle layers fuse and thicken with the development of diastolic HF, whereas ordered laminar arrangement of myocytes is disrupted and heterogeneous patchy fibrosis develops.99 In addition to this perimysial collagen remodeling, endomysial collagen surrounding individual myocytes thickens substantially.99,100 Finally, end-stage systolic failure is associated with ventricular dilation. These factors
increase the probability that ectopic activation will occur and that reentrant VT will be developed and sustained.

In one of the most comprehensive recent studies in this area, Glukhov et al mapped transmural electric activation in coronary-perfused LV wedges from normal human hearts acquired for transplantation and from explant hearts in end-stage failure. Regional molecular and structural remodeling were characterized in the same hearts. HF resulted in marked fibrosis and Cx43 remodeling at all of the sites across the LV wall. Total Cx43 associated with N-cadherins was reduced 3-fold to 4-fold, and the ratio of dephosphorylated to phosphorylated Cx43 was increased. APD was prolonged and more uniform transmurally in HF than in normal hearts, whereas APD alternans was induced by pacing at increased rates in HF but not in normal preparations. Finally, axial CV was unchanged in HF (Supplement in Glukhov et al), but transverse CV was markedly reduced and exhibited steeper restitution. That is, electric anisotropy was increased in HF, giving rise to slower and more rate-dependent transmural activation spread.

Very similar findings were reported for isolated human hearts in end-stage HF, as well as isolated hearts from senescent mice and mice after thoracic aortic banding. In these studies, CVs and activation delays were estimated using high-resolution extracellular epicardial mapping; axial CV was unchanged, whereas significant rate-dependent transverse activation delays were seen. The probability of inducing VT and fibrillation in these hearts was increased by the presence of patchy fibrosis. Furthermore, inhibition of the renin-angiotensin-aldosterone system in aging mice limited age-related fibrosis, decreased the extent of electric anisotropy, and lowered arrhythmogeneity.

The preservation of axial CV in HF suggests that gap junction remodeling and fibroblast/myofibroblast infiltration have relatively little impact on this parameter. The decreased transverse CV in HF has been linked with Cx43 remodeling. However, a more direct explanation of the increased electric anisotropy seen in aging hearts, hypertensive heart disease, and HF is that it reflects changes in the 3D connective tissue matrix. Remodeling of the perimysial collagen network and the disruption of ordered branching between muscle layers would be expected to impair transverse activation spread. Likewise, thickening of the endomysial collagen network could impact side-to-side coupling at the cellular level. Thus, the structural substrate for reentry has features in common with the infarct BZ (tortuous conduction and source-to-sink mismatch), although here (like the atria in AF) the heterogeneities are more widely and more diffusely distributed throughout the myocardium. As discussed previously, direct electronic interactions between the fibroblast/myofibroblast network and myocytes may also contribute to arrhythmia in this setting.

The rate dependence of cellular electric instability in HF is of considerable interest. Repolarization alternans (T-wave alternans) predicts sudden cardiac death in HF, in particular at low heart rates when APD restitution slope is relatively flat. There is also a strong association between the distribution or texture of fibrosis and risk of arrhythmia.
Computer modeling suggests that the risk of wavebreak and fibrillation is increased by structural discontinuities across a range of spatial scales, fibrosis is more likely to give rise to reentry and fibrillation if it is patchy and nonuniformly distributed, and fibrosis can amplify regional rhythm instability because of its effects on local propagation and electrotonic current flow. These issues need to be investigated using models that more accurately represent the 3D arrangement of myocytes and fibrosis throughout the progression of HF.

Conclusions

All of the models, whether conceptual, experimental, in vitro, or in silico, are, by definition, simplifications of reality, and some may distort reality. It is necessary to be aware of these limitations when seeking to relate model outcomes to the real world. The most successful models are those that explain the widest range of physical evidence with least complexity and fewest assumptions.

In this article, we have reviewed the mechanisms that determine 3D impulse propagation in the heart with particular emphasis on structure at the tissue level. It is necessary to account for electric anisotropy and structural heterogeneities at multiple scales to make sense of existing data relating to 3D impulse propagation in the normal heart and in heart disease. For example, many of the features that characterize electric activation in structural heart disease, anisotropic impulse propagation, rate-dependent activation delays, and electric instability can be reproduced in tissue level models based on structural remodeling alone. In addition to cellular and molecular remodeling, the influence of structural remodeling on electric activation is also important in this setting.

In preparing this review, we have been reminded of the enormous progress made in the biophysical understanding of heart rhythm disturbance over the past 30 years. Computer modeling in cardiac electrophysiology has come of age during this period, and it is now an essential tool in this discipline. However, the linkage between extent and distribution of fibrosis and risk of reentrant arrhythmia in structural heart disease requires detailed further investigation, and the mechanisms that underlie persistent AF remain unclear. To address these issues, we need a more quantitative understanding of the ways in which cellular, molecular, and structural remodeling alter 3D impulse propagation in the myocardium. The development of biophysically based models, which capture the roles of these factors at the tissue level, is a necessary step toward this objective. However, complementary 3D imaging methods also will be required, together with new experimental techniques that allow for robust validation of model predictions.

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