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Effects of Fibroblast-Myocyte Coupling on Cardiac Conduction and Vulnerability to Reentry: A Computational Study

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Abstract

Background—Recent experimental studies documented that functional gap junctions form between fibroblasts and myocytes, raising the possibility that fibroblasts play roles in cardiac electrophysiology that extend beyond acting as passive electrical insulators.

Objectives—Using computational models, we investigated how fibroblasts may affect cardiac conduction and vulnerability to reentry under different fibroblast-myocyte coupling conditions and tissue structures.

Methods—Computational models of two-dimensional tissue with fibroblast-myocyte coupling were developed and numerically simulated. Myocytes were modeled by the Phase I of the Luo-Rudy model and fibroblasts by a passive model.

Results—Besides slowing conduction by cardiomyocyte decoupling and electrotonic loading, fibroblast coupling to myocytes elevates myocyte resting membrane potential, causing conduction velocity to first increase and then decrease as fibroblast content increases, until conduction failure occurs. Fibroblast-myocyte coupling can also enhance conduction by connecting uncoupled myocytes. These competing effects of fibroblasts on conduction give rise to different conduction patterns under different fibroblast-myocyte coupling conditions and tissue structures. Elevation of myocyte resting potential due to fibroblast-myocyte coupling slows sodium channel recovery, which extends post-repolarization refractoriness. Due to this prolongation of myocyte refractory period, reentry was more readily induced by a premature stimulation in heterogeneous tissue models when fibroblasts were electrotonically coupled to myocytes compared to uncoupled fibroblasts acting as pure passive electrical insulators.

Conclusions—Fibroblasts affect cardiac conduction acting as obstacles, or by creating electrotonic loading and elevating myocyte resting potential. Functional fibroblast-myocyte coupling prolongs myocyte refractory period which may facilitate induction of reentry in cardiac tissue with fibrosis.

Keywords

Fibrosis; Fibroblasts; Conduction; Reentry; Vulnerability

INTRODUCTION

Fibrosis, characterised by fibroblast proliferation and collagen production, is associated with an increased risk of arrhythmias in many cardiac diseases and aging^{1, 2}. The underlying mechanism has traditionally been attributed to fibrosis causing cardiomyocyte decoupling, predisposing the tissue to conduction block and reentry^{3, 4}. However, recent experimental studies have documented that fibroblasts express connexins, and that functional gap junctions can form between myocytes and fibroblasts *in vitro*^{5–7} and *in situ*^{8–10}, although the extent to which this occurs in normal and pathologically disturbed myocardium is still unknown. Confirmation of fibroblast-myocyte coupling raises the possibility that fibroblasts may play active roles in cardiac arrhythmogenesis, beyond acting as passive electrical insulators.

Direct electrophysiological effects of fibroblast-myocyte coupling have been investigated in experimental studies mainly using cell pairs^{11, 12} and cultured monolayers^{6, 7, 13–16} and three-dimensional (3D) constructs¹⁷ as well as in research using computational models^{14, 18–25}. In cultured rat neonatal ventricular myocyte strands, Gaudesius et al.⁶ showed that electrical activity could be conducted over a distance of up to 300µm via fibroblasts, causing insert-length dependent delays in wave propagation. In co-cultures of myocytes and fibroblasts, Miragoli et al.¹³ showed that conduction velocity (CV) first increased and then decreased as the fibroblast density was raised. Zlochiver et al.¹⁴ demonstrated that CV responded non-monotonically when gap junction coupling of myofibroblasts was reduced by siRNA, or increased by overexpression of Cx43. It has also been shown that fibroblast-myocyte coupling can depolarize myocyte membrane potential sufficiently to facilitate automaticity¹⁵. These experimental studies provide direct evidence that myocytes and fibroblasts can be coupled via gap junctions, and that this is a commonplace occurrence when co-cultured. There is experimental evidence for direct fibroblast-myocyte coupling in pacemaker tissue⁹ and infarct scars¹⁰, but the extent to which this may occur in normal working myocardium, and how this may change during development or pathogenesis is currently unknown.

Computational studies have explored the effects of several different fibroblast-myocyte coupling configurations. Kohl and Noble²⁶ simulated a sino-atrial node pacemaker cell coupled to a fibroblast and showed that the fibroblast increased the spontaneous pacemaker rate. Jacquemet and Henriquez^{18, 19} simulated a two-dimensional (2D) sheet of myocytes with fibroblasts attached randomly to (but not inserted between) myocytes. They showed that CV first increased and then decreased, as the gap junction conductance between myocytes and fibroblasts increased. Zlochiver et al.¹⁴, on the other hand, simulated coupled fibroblasts inserted into a 2D layer of myocytes and showed that CV first decreased and then increased as the gap junction conductance increased, in agreement with their experimental observations. Sachse et al also studied the effects of fibroblasts, inserted between myocytes, on CV in a one-dimensional monodomain cable²⁰ and in a 2D bidomain model²¹. Fibroblast-myocyte coupling also has been shown to affect action potential duration (APD) in computational studies^{19, 20, 23, 27}, either prolonging or shortening APD depending on the resting potential of isolated fibroblasts. Tanaka et al.²² and Zlochiver et al.¹⁴ showed that different fibroblast distributions affected wavebreaks in 2D tissue models.

Despite the increasing number of experimental and modeling studies, a unified picture of how fibroblasts affect conduction and promote wavebreak has not yet emerged. For example, based on their experimental observations, Miragoli et al.¹³ hypothesized that the nonmonotonic CV changes were due to elevation of myocyte resting potential by fibroblasts, similar to the well known effect of increased extracellular potassium concentration $[K^+]_o$.^{28, 29} Jacquemet and Henriquez¹⁹, on the other hand, attributed the nonmonotonic CV changes to electrical loading effects of fibroblasts in their computational model. Zlochiver et al.¹⁴ interpreted their similar observations as resulting from competition between conduction slowing by cellular decoupling causing zigzag pathways and fibroblast-myocyte coupling facilitated conduction.

In this study, we used computational modeling to quantitatively assess the impact of fibroblast-myocyte coupling on conduction and arrhythmogenesis in cardiac tissue with different types of fibroblast-myocyte coupling as proposed by Kohl and Camelliti³⁰, i.e., zero-sided connection, single-sided connection, and double-sided connection. In zero-sided connection, fibroblasts are inserted in myocardial tissue but they do not form gap junctions with myocytes (equivalent to obstacles). In single-sided connection, a fibroblast couples to one or more myocytes that are themselves in direct electrical contact, but does not couple to another “electrically distant” myocyte to “bridge” them. In double-sided connection, a fibroblast or group of fibroblasts is inserted between myocytes that are not themselves in direct electrical contact, and electrically bridges these originally uncoupled myocytes. We simulated these types of fibroblast-myocyte coupling in 2D monodomain models to delineate how their structural and electrical effects alter conduction by creating obstacles, electrotonic loading, and elevating myocyte resting potential. We also examined vulnerability to reentry in these tissue models, and find that fibroblast-myocyte coupling facilitates the induction of reentry beyond the level observed with zero-sided ‘barriers’, by prolonging the effective refractory period.

METHODS

Fibroblast-myocyte coupling

The membrane voltage (V) of a myocyte (V_m) or a fibroblast (V_f) in the tissue models is governed by:

$$C \frac{dV}{dt} = -I_{ion} + \sum_{k=1}^n G_{gap}^k (V^k - V) \quad (1)$$

where C is the membrane capacitance of a myocyte (C_m) or a fibroblast (C_f), I_{ion} is the corresponding membrane current (I_m or I_f). n is the number of coupled neighbors (either myocytes or fibroblasts). G_{gap}^k is the gap junction conductance between a cell (either a myocyte or a fibroblast) and its k^{th} neighbor (either a myocyte or a fibroblast). The size of the myocyte was set to $125 \times 25 \times 25 \mu m$, with $C_m = 125$ pF. I_m was formulated from a modified version (see Supplemental Materials for details) of the LR1 model³¹. The size of the fibroblast was set to $25 \times 25 \times 25 \mu m$. For the membrane current of the fibroblast, we used a “passive” model³², i.e.,

$$I_f = G_f (V - E_f) \quad (2)$$

where G_f is the membrane conductance and E_f the resting potential. Although many of the electrophysiological properties of the fibroblast are still unknown, it was estimated based on experiments that C_f ranges from 6.3 to 75 pF^{23, 33}, G_f ranges from 0.1 to 4 nS³², and E_f ranges from -50 to 0 mV^{8, 12, 34–36}. The gap junction conductance between myocyte and fibroblast or between two fibroblasts is denoted as G_j in this paper. Rook et al.¹² reported that G_j ranges from 0.3 to 8 nS in cultured cells, whereas $0 < G_j < 100$ nS were used in other modeling studies^{19, 20, 23}. In this study, we used $C_f=25$ pF, $E_f=-20$ mV, and $G_j=20$ nS as the default set of parameters, and specified whenever changes from the default values were made.

Tissue models

For the 2D tissue models, we incorporated cells into tissue using a random brick wall pattern. The random brick wall gives rise to a slightly larger CV than the uniform model, as shown by Hubbard et al.³⁷ The tissue structures and fibroblast distributions are described in detailed in Supplemental Materials. The gap junction conductance between myocytes was set to 600 nS when two myocytes were coupled end-to-end, and 1000 nS when they fully overlapped side-to-side. When two myocytes were partially overlapping side-to-side, gap junction conductance was proportional to the degree of overlap. This gave rise to a longitudinal CV of 0.56 m/s and a transverse CV of 0.13 m/s in the absence of fibroblasts.

The details of APD and CV measurements and numerical methods are described in Supplemental Materials.

RESULTS

Fibroblasts modulate conduction velocity and failure

Although previous studies have characterized the effects of either fibroblast attachment¹⁹ or insertion^{14, 20, 21} on action potential conduction, a unified picture of how fibroblasts affect the propagation of excitation is lacking. Here we analyzed systematically the mechanisms by which fibroblasts affect conduction in different tissue structures with various types of fibroblast-myocyte couplings³⁰.

Random fibroblast attachment on top of a myocyte 2D sheet—We first studied the effects of single-sided fibroblast-myocyte connections on conduction using a model similar to Jacquemet and Henriquez¹⁹, in which fibroblasts are randomly attached to (but not inserted between) myocytes that themselves form a 2D sheet (Fig. 1A; note that there is no point in simulating the zero-sided case for what would be essentially a parallel but unconnected sheet of fibroblasts). We paced the tissue at one end and calculated the average CV for different fibroblast-myocyte (F-M) ratios and different fibroblast properties. With E_f set to -20 mV with small fibroblast membrane conductances, G_f , (filled circles in Fig. 1B), CV remained initially almost unchanged as the F-M ratio increased, but then decreased quickly as the F-M ratio approached 3:1. Conduction failed at an F-M ratio of 3.35:1. When G_f was large (triangles and diamonds), however, CV increased initially and then decreased, with conduction failure at lower F-M ratios. In all three cases, conduction failed after CV decreased to around 0.2 m/s. This biphasic effect is similar to the biphasic effects of depolarizing myocyte resting potential with increased extracellular potassium concentration $[K^+]_o$ ^{28, 29}. The mechanism is that elevation of V_{mr} first increases CV by bringing the myocyte closer to the threshold of I_{Na} , but then decreases CV as I_{Na} is progressively inactivated. To quantitatively compare the effects by the fibroblast-myocyte coupling and by $[K^+]_o$ elevation, we plot the relationship between CV and V_{mr} versus $[K^+]_o$ from a one-dimensional cable without fibroblasts in Fig. 1C. As $[K^+]_o$ increases, V_{mr} increases monotonically, whereas CV first increases and then decreases. Conduction failure occurred

when $[K^+]_o$ was elevated to 13 mM, at which point the resting membrane potential was about -64 mV (indicated by the dashed arrows). The relationship between V_{mr} and the F-M ratio from a myocyte coupled with fibroblasts in Fig. 1D shows that using -64 mV as the threshold of conduction failure (the dashed line), the predicted F-M ratios for conduction failure (arrows) agree with the results in Fig. 1B.

The effects of F-M ratio on CV depend on E_f ¹⁹. As E_f decreases, the effect of fibroblasts on elevation of myocyte resting potential (V_{mr}) is reduced and so is the effect on CV. When E_f is the same as the resting potential of the isolated myocyte, fibroblasts have no effect on the value of the myocyte resting potential. In this case, a fibroblast represents a purely passive electrotonic load to a myocyte during the action potential upstroke phase, since its membrane capacitance needs to be charged by the myocyte. To explore this effect, we set E_f to -80 mV, which is very close to the resting potential of the myocyte and has almost no effect on the V_{mr} . Under this condition, CV decreased linearly from 0.56 m/s to 0.49 m/s as F-M ratio increased from 0 to 3:1, almost independent of the value of G_f (Fig. 1E).

Random fibroblast insertion in series with myocytes in a 2D sheet—In this model, fibroblasts were inserted randomly in series between myocytes to break the normal myocyte-myocyte coupling (Fig. 2A). The effects of zero-sided connection (uncoupled fibroblasts) and double-sided connection (fibroblasts coupled to their neighboring myocytes and/or fibroblasts) on conduction were studied. Uncoupled fibroblasts serve as obstacles which decouple in-line myocytes, but allow local “zigzag” conduction around them through micro-branching pathways. In this case, CV progressively decreased as the F-M ratio increased (black squares in Fig. 2B), with conduction failure occurring at F-M ratios above 3:1. When fibroblasts with $E_f = -20$ mV were coupled to myocytes, for small membrane conductance G_f , CV was almost the same as in the case of uncoupled fibroblasts, and conduction failure occurred at a similar F-M ratio (black dots in Fig. 2B). For larger G_f , CV decreased much faster as the F-M ratio increased, and conduction failure occurred at lower ratios (triangles and diamonds in Fig. 2B), similar to those in Fig. 1B. The conduction failure at large G_f was due primarily to the elevation in myocyte resting potential caused by coupled fibroblasts (Fig. 2C). When we used $E_f = -80$ mV, CV was only slightly different from uncoupled fibroblasts, and almost independent of G_f (Fig. S1), with the difference attributed to pure electrotonic loading effects. Note that, as may be expected, randomly *inserted* (in series) fibroblasts, whether coupled or not, caused a much faster decrease in CV as F-M ratio increased (Fig. 2B) than randomly *attached* (‘on top’) fibroblasts (Fig. 1B), and CV decreased monotonically. This is because the inserted fibroblasts result in “zigzag” pathways and micro-branching structures in the tissue which slows CV, as shown previously³⁸, while attached fibroblasts merely act as an electrotonic load. For our parameter settings, the action potential cannot conduct through a myocyte-fibroblast-myocyte pathway ($G_j > 120$ nS is needed for such conduction, see Online Fig. S2A) and thus can only conduct through myocyte-myocyte pathways, which forms the zigzag pathways and branching structures. Even for $G_j > 120$ nS in which the action potential can conduct through a myocyte-fibroblast-myocyte coupling, the conduction delay (Fig. S2B) is much longer than conduction through a myocyte-myocyte-myocyte coupling (~ 0.5 ms in our system), so that the action potential still conducts through the available alternative myocyte-myocyte pathways in the tissue.

However, G_j also affects CV and conduction failure. For a fixed G_f , conduction tended to fail at lower F-M ratios for higher G_j (Fig. 2D). For a fixed F-M ratio, CV changed nonmonotonically with G_j (Fig. 2E). For an F-M ratio at 1:1 and $G_f = 2$ nS, CV first increased to a maximum, then decreased to a minimum, and eventually increased linearly ($G_j > 25$ nS) as G_j increased. For $G_f = 0.1$ nS, CV decreased first and then increased linearly ($G_j > 25$ nS) as G_j increased. In both experiments and simulations, Zlochiver et al.¹⁴ showed that CV decreased to a minimum and then increased as G_j increased, which agrees with the lower G_f

case in Fig. 2E. These behaviors can be understood as follows. The myocyte resting potential V_{mr} increases as G_j increases (Fig. S3A) which is mild when G_f is small. Therefore, when G_f is large, the effects of V_{mr} elevation predominates over the electrotonic loading effect, so that CV increase. However, as G_j increases further, the effect on V_{mr} saturates while the electrotonic effect increases continuously, which causes CV to decrease. When G_f is small, the loading effect predominates, so that CV decreases as G_j increases. In both cases, when $G_j=0$, the fibroblasts are simply obstacles which slow conduction (to 0.33 m/s in Fig. 2E), but as G_j increases, depolarization of myocytes by fibroblasts speeds conduction. When G_j becomes large, this effect predominates over the electrotonic loading effect to increase CV. This later increasing phase of CV does not occur in the attachment model (Fig. S3B), as also shown by Jacquemet and Henriquez¹⁹.

Random fibroblast insertion in parallel with myocytes in a 2D sheet—

Immunohistological analysis of cardiac tissue shows that fibroblasts preferentially reside in the plane of myocardial tissue, along the lateral sides of myocytes (Fig. 3A), rather than between end-to-end contacts. Accordingly, we simulated tissue in which fibroblasts were connected laterally between, but in the plane of, myocytes (Fig. 3B). Without fibroblasts present, CV in the longitudinal and transverse directions were 0.55 m/s and 0.155 m/s, respectively. With random laterally inserted fibroblasts coupled to all neighboring cells (double-sided connection), CV changed in both directions, but to a different extent. In the longitudinal direction, CV properties were similar to models with random fibroblast attachment (as in Fig. 1), with minor differences due to micro-branching effects (Fig. 3C). In the transverse direction (Fig. 3D), however, CV decreased much more rapidly, more similar to the model of random fibroblast insertion shown in Fig. 2.

Fibroblasts in laminar clefts—It has been shown that cardiac tissue exhibits laminar structures (left panel in Fig. 4A)³⁹, with fibroblasts tending to localize in the cleft spaces (right panel in Fig. 4A)⁸. To study how fibroblasts affect conduction in this type of structure, we created a 2D model in which 2 to 4 rows of myocytes were separated by random clefts with a mean longitudinal length of 1.25 mm, with fibroblasts randomly positioned in these clefts (Fig. 4B). The fibroblasts were coupled to myocytes at both sides of the cleft (double-sided connection). In the longitudinal direction, myocytes remained aligned end-to-end and therefore retained their well-coupled properties. This model could be taken to simulate a 2D section in the ‘cross-sheet’ plane, while the previous models would be ‘in-sheet’ models of myocyte-fibroblast interactions. As fibroblast content increased, CV decreased monotonically for $G_f=1$ nS, while CV increased and then decreased until conduction failure for $G_f=4$ nS (Fig. 4C). This can be explained by depolarization of myocyte resting potential and electrotonic loading.

In the ‘trans-laminar’ direction, however, as fibroblast content increased, CV increased monotonically for $G_f=1$ nS, while CV increased and then decreased until conduction failure for $G_f=4$ nS (Fig. 4D). Fibroblasts enhanced conduction for low G_f in the trans-laminar direction, possibly via narrow pathways (like bridges) in the transverse direction which slow conduction or fail to conduct (due to source-sink effect). When the randomly placed fibroblasts are coupled to myocytes near these narrow pathways, they change the source-sink relation to facilitate conduction. In addition, although the action potential cannot directly conduct through the fibroblasts in the cleft for the G_j we used, depolarization of the fibroblasts by myocytes from one side of the cleft will help depolarization of myocytes in the other side, which may also enhance conduction, similar to enhance conduction by increasing G_j in Fig. 2E.

Fibroblast-myocyte coupling and vulnerability to reentry

The arrhythmogenic effects of fibrosis are generally attributed to cellular decoupling, promoting slow conduction and facilitating block. Here we studied how fibroblast-myocyte coupling in different cardiac tissue models affects induction of reentry.

Diffuse regional fibroblast distribution—To study the effects of fibroblast-myocyte coupling on vulnerability to reentry by a single premature stimulation, we simulated a 5 cm × 1.5 cm tissue model with a central fibrotic region, with either randomly attached (single-sided ‘piggy-back’ connection) or randomly inserted (zero- or double-sided ‘in-plane’ connections) fibroblasts. In the case of randomly attached fibroblasts with an F-M ratio of 1.5 in the central fibrotic region, CV of the S1 beat was not affected by the presence of fibroblasts (see the wavefront in the 2nd panel of Fig. 5A), but a premature “S2” beat blocked in the central fibrotic region and induced reentry (Fig. 5A). Reentry could only be induced when the coupling strength G_j between fibroblasts and the myocytes exceeded a critical value (Fig. 5B). The S1S2 interval initiating reentry increased as G_j increased, reaching a plateau for very large G_j . The vulnerable window also initially increased as G_f increased (Fig. 5C) or the F-M ratio increased (Fig. 5D). For very large G_f or F-M ratios, however, vulnerability decreased.

In the case of random fibroblast insertion, CV in the central fibrotic region was substantially slower (see the wavefront in the 2nd panel in Fig. 6A and the CV shown in Fig. 2B). When fibroblasts were uncoupled, conduction slowed, but reentry could not be induced for any F-M ratio. When fibroblasts were coupled with myocytes, reentry could be induced by an S2 (Fig. 6A) when G_j and F-M ratio reached certain critical values. Compared to the case of random fibroblast attachment (Fig. 5), the vulnerable windows were similar, but reentry occurred at a lower G_j (Fig. 6B), lower G_f (Fig. 6C), and over a narrower F-M ratio range (Fig. 6D).

Although CV was substantially slowed by fibroblasts inserted in-plane (compared to piggy-back attached ones), the vulnerable windows for reentry were nearly the same. This is because the common factor causing conduction block in both cases was the prolongation of the effective refractory period (ERP) due to post-repolarization refractoriness, rather than conduction slowing *per se*. To demonstrate this, we quantitatively compared the change in ERP with the vulnerable window. ERP, defined as the minimum S1S2 interval for which an action potential can be elicited by an S2 (Fig. 7A), was determined in single myocytes at different F-M ratios. In Fig. 7B–D, we plot Δ APD, Δ ERP, and Δ S1S2 (see definition in legend) for reentry from Figs. 5 and 6. The Δ S1S2 correlate well with Δ ERP, but not Δ APD. Fibroblasts prolong ERP much more than they prolong APD because elevation of resting potential by fibroblasts slows the recovery of the Na current (see Fig. S4). However, marked prolongation of regional ERP eventually decreases vulnerability to reentry as shown previously⁴⁰, which explains why vulnerable window decreased for large G_f or F-M ratio in both Figs. 5 and 6.

Coupled fibroblasts bridging laminar clefts—When coupled fibroblasts were inserted into the laminar clefts separating layers of myocytes, vulnerability to reentry increased, despite facilitation of conduction in the trans-laminar direction by the inserted fibroblasts. To examine how fibroblasts increase vulnerability, we first studied conduction block in a model where two layers of myocytes, separated by a cleft, were interconnected centrally by a myocyte bridge (4 myocytes wide and 2 myocytes long, Fig. 8A). In this case, conduction following a premature S2 stimulus was blocked in the bridge at S1S2 coupling intervals from 270 ms to 334 ms. After attaching 20 fibroblasts uniformly to the 8 myocytes forming the bridge, however, the range of S1S2 intervals during which the premature S2

beat was blocked was increased, ranging from 270 ms to 436 ms. Note that attaching fibroblasts to the bridge may even enhance conduction across the bridge (as in Fig. 4D). The prolongation of ERP due to fibroblast-myocyte coupling resulted in the widening of the S1S2 interval for uni-directional block. However, for this uni-directional conduction block to cause reentry around a single cleft, an unrealistically long cleft would be needed (cleft length > 10 cm in the model, results not shown).

Alternatively, we also simulated a tissue structure with many short clefts (Fig. 8B). In the absence of coupled fibroblasts, the structural heterogeneity imposed by the clefts caused zigzag conduction, but reentry could not be induced by premature S2 beats (Fig. 8C). When fibroblasts were randomly inserted into the clefts and coupled with the neighboring myocytes, reentry could be induced when F-M ratio exceeded a critical value (Fig. 8D). The CV in the cleft area after fibroblasts insertion (0.227 m/s) was almost the same as that of no fibroblasts (0.226 m/s). However, insertion of fibroblasts to the clefts prolonged the ERP in the cleft area, and thus increased the vulnerability to reentry, the same mechanism of reentry induction as shown in Figs. 5 and 6.

DISCUSSION

Mechanisms by which fibroblasts modulate cardiac conduction

In this study, we investigated the mechanisms by which fibroblasts affect conduction for different fibroblast-myocyte couplings configurations and tissue structures. We showed that: 1) fibroblast-myocyte coupling elevates myocyte resting potential, resulting in a biphasic change in CV; 2) fibroblasts also slow conduction by their electrotonic loading effects; 3) inserted fibroblasts, whether coupled to myocytes or not, slow conduction by creating “zigzag” conduction pathways; 4) fibroblast-myocyte coupling can enhance conduction in laminar tissue structures. In intact cardiac tissue or cultured monolayers, fibroblasts may fill in the interstitial spaces, break existing myocyte-myocyte couplings, and couple with their neighboring myocytes in different ways³⁰. Therefore, all the mechanisms we show in this study may be applicable in fibrotic tissue or co-cultured monolayers, which may compete to give rise to non-monotonic CV changes with increasing fibroblast content or coupling strength.

In an experimental study in co-cultured monolayers, Miragoli et al.¹³ found a biphasic effect on CV when endogenous fibroblasts proliferated with the myocytes (equivalent to the insertion model), but not when fibroblasts were plated on top of myocytes (equivalent to the attachment model). This seems opposite to the simulation results in the present and previous studies^{14, 19–21} comparing insertion and attachment. Although the exact cause is not clear to us, one possible explanation is the following. In the endogenous fibroblast case, fibroblasts proliferate in the interstitial spaces and couple to neighboring myocytes. In this situation, fibroblasts first increase CV by elevating the resting potential (as in Fig. 1B) and by bridging myocytes through double-sided connections (as in Fig. 4D). As fibroblast content increases further, fibroblasts then slow CV by breaking the existing myocyte-myocyte couplings (as in Fig. 2B) and by further elevating resting potential (as in Fig. 1B). However, in the case of exogenous fibroblasts plated on top of a pre-formed myocyte monolayer, one possibility is that endogenous fibroblast content might be high enough to obscure the increasing CV phase as exogenous fibroblast content increases. Of course, the potential contributions of other mechanisms, such as paracrine interactions, cannot be assessed with the given electrophysiological models.

Implications for arrhythmogenesis in fibrosis

Fibrosis, a key mediator of structural remodeling in cardiac disease, has been closely linked to cardiac arrhythmias^{1, 2}. The traditional concept is that cellular decoupling due to fibrosis causes slow and/or fractionated conduction, predisposing the heart to reentrant arrhythmias. However, modeling studies showed that pure cellular decoupling⁴⁰ barely increases vulnerability to reentry. Here we demonstrate that when fibroblasts are coupled to myocytes, vulnerability to reentry by a premature extrasystole is substantially potentiated. The underlying mechanism is that fibroblast-myocyte coupling elevates myocyte resting potential, resulting in post-repolarization refractoriness in fibrotic regions, which can facilitate local wave block initiating reentry. Thus, fibroblast-myocyte coupling may be a key contributor to the increased arrhythmia risk in fibrotic hearts.

Note that in normal hearts, fibroblasts comprise the majority of the non-myocyte cells, accounting for up to 2/3 of cell numbers^{8, 41}. Direct experimental evidence on fibroblast-myocyte coupling is scarce in intact working myocardium (available evidence suggests electrophysiologically-relevant contributions from fibroblasts in pathophysiological conditions¹⁰ or specialized tissue such as the sino-atrial node⁹). Based on our simulation results (Figs. 5 and 6), the number of fibroblasts coupled to the myocytes, the gap junction conductance, and the fibroblast membrane capacitance have to exceed certain values to be arrhythmogenic. These conditions may not be satisfied in normal conditions, but may occur in diseased hearts. For example, in a recent study, Vasquez et al⁴² showed that cardiac fibroblasts isolated from normal hearts grown under standard tissue culture expressed significantly less Cx40 and Cx43 than those isolated from ischemic hearts grown under the same conditions. This suggests that fibroblasts may be ‘electrophysiologically invisible’ in the normal heart but arrhythmogenic in ischemic tissue in which fibroblast-myocyte coupling may be strong enough to surpass the coupling strength threshold for reentrant arrhythmias. This would be in keeping with similar findings in native post-infarct tissue⁴³.

Limitations

Several limitations of this study should be acknowledged. First, we used a passive membrane model of fibroblast³² in order to be able to freely and independently adjust the parameters E_f and G_f . However, a passive model does not accurately represent all of the electrophysiological features of fibroblasts. Although different fibroblasts models have been developed^{18, 20, 23}, due to the limited availability of detailed electrophysiological data on fibroblasts, and the technical difficulty to record from cells with membrane resistances in the $G\Omega$ region, the available models are largely phenomenological. A second major limitation relates to simulating laminar sheet structures using a 2D model, which may obscure potentially important 3D trans-fiber effects of the laminar clefts. A real 3D structure is needed for further study, ideally modeled on a realistic representation of 3D cell distribution and coupling. In the absence of such detailed data, our study provides general mechanistic insight into how the configuration of fibroblast-myocyte coupling in different tissue structures may affect cardiac conduction, potentially facilitating reentry, which may explain some of the proarrhythmic effects of fibroblasts in pathophysiological settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Brown RD, Ambler SK, Mitchell MD, et al. The Cardiac Fibroblast: Therapeutic Target in Myocardial Remodeling and Failure. *Annu Rev Pharmacol Toxicol* 2005;45:657–687. [PubMed: 15822192]
2. Burstein B, Nattel S. Atrial Fibrosis: Mechanisms and Clinical Relevance in Atrial Fibrillation. *J Am Coll Cardiol* 2008;51:802–809. [PubMed: 18294563]
3. de Bakker JMT, van Rijen HMV. Continuous and Discontinuous Propagation in Heart Muscle. *J Cardiovasc Electrophysiol* 2006;17:567–573. [PubMed: 16684038]
4. Spach MS, Heidlage JF, Dolber PC, et al. Mechanism of origin of conduction disturbances in aging human atrial bundles: Experimental and model study. *Heart Rhythm* 2007;4:175–185. [PubMed: 17275753]
5. Goshima K. Formation of nexuses and electrotonic transmission between myocardial and FL cells in monolayer culture. *Exp Cell Res* 1970;63:124–130. [PubMed: 5531475]
6. Gaudesius G, Miragoli M, Thomas SP, et al. Coupling of cardiac electrical activity over extended distances by fibroblasts of cardiac origin. *Circ Res* 2003;93:421–428. [PubMed: 12893743]
7. Chilton L, Giles WR, Smith GL. Evidence of intercellular coupling between co-cultured adult rabbit ventricular myocytes and myofibroblasts. *J Physiol* 2007;583:225–236. [PubMed: 17569734]
8. Camelliti P, Borg TK, Kohl P. Structural and functional characterisation of cardiac fibroblasts. *Cardiovasc Res* 2005;65:40–51. [PubMed: 15621032]
9. Camelliti P, Green CR, LeGrice I, et al. Fibroblast network in rabbit sinoatrial node: structural and functional identification of homogeneous and heterogeneous cell coupling. *Circ Res* 2004;94:828–835. [PubMed: 14976125]
10. Walker NL, Burton FL, Kettlewell S, et al. Mapping of epicardial activation in a rabbit model of chronic myocardial infarction. *J Cardiovasc Electrophysiol* 2007;18:862–868. [PubMed: 17537208]
11. Rook MB, Jongsma HJ, de Jonge B. Single channel currents of homo- and heterologous gap junctions between cardiac fibroblasts and myocytes. *Pflugers Arch* 1989;414:95–98. [PubMed: 2471143]
12. Rook MB, van Ginneken AC, de Jonge B, et al. Differences in gap junction channels between cardiac myocytes, fibroblasts, and heterologous pairs. *Am J Physiol Cell Physiol* 1992;263:C959–977.
13. Miragoli M, Gaudesius G, Rohr S. Electrotonic modulation of cardiac impulse conduction by myofibroblasts. *Circ Res* 2006;98:801–810. [PubMed: 16484613]
14. Zlochiver S, Munoz V, Vikstrom KL, et al. Electrotonic Myofibroblast-to-Myocyte Coupling Increases Propensity to Reentrant Arrhythmias in Two-Dimensional Cardiac Monolayers. *Biophys J* 2008;95:4469–4480. [PubMed: 18658226]
15. Miragoli M, Salvarani N, Rohr S. Myofibroblasts induce ectopic activity in cardiac tissue. *Circ Res* 2007;101:755–758. [PubMed: 17872460]
16. Fahrenbach JP, Mejia-Alvarez R, Banach K. The relevance of non-excitabile cells for cardiac pacemaker function. *J Physiol* 2007;585:565–578. [PubMed: 17932143]
17. Baudino TA, McFadden A, Fix C, et al. Cell patterning: interaction of cardiac myocytes and fibroblasts in three-dimensional culture. *Microsc Microanal* 2008;14:117–125. [PubMed: 18312716]
18. Jacquemet V, Henriquez CS. Modelling cardiac fibroblasts: interactions with myocytes and their impact on impulse propagation. *Europace* 2007;9(Suppl 6):vi29–37. [PubMed: 17959690]
19. Jacquemet V, Henriquez CS. Loading effect of fibroblast-myocyte coupling on resting potential, impulse propagation, and repolarization: insights from a microstructure model. *Am J Physiol Heart Circ Physiol* 2008;294:H2040–2052. [PubMed: 18310514]
20. Sachse FB, Moreno AP, Abildskov JA. Electrophysiological modeling of fibroblasts and their interaction with myocytes. *Ann Biomed Eng* 2008;36:41–56. [PubMed: 17999190]
21. Sachse F, Moreno A, Seemann G, et al. A Model of Electrical Conduction in Cardiac Tissue Including Fibroblasts. *Ann Biomed Eng* 2009;37:874–889. [PubMed: 19283480]

22. Tanaka K, Zlochiver S, Vikstrom KL, et al. Spatial Distribution of Fibrosis Governs Fibrillation Wave Dynamics in the Posterior Left Atrium During Heart Failure. *Circ Res* 2007;101:839–847. [PubMed: 17704207]
23. MacCannell KA, Bazzazi H, Chilton L, et al. A mathematical model of electrotonic interactions between ventricular myocytes and fibroblasts. *Biophys J* 2007;92:4121–4132. [PubMed: 17307821]
24. Maleckar MM, Greenstein JL, Trayanova NA, et al. Mathematical simulations of ligand-gated and cell-type specific effects on the action potential of human atrium. *Prog Biophys Mol Biol* 2008;98:161–170. [PubMed: 19186188]
25. Jacquemet V, Henriquez CS. Modulation of conduction velocity by nonmyocytes in the low coupling regime. *IEEE Trans Biomed Eng* 2009;56:893–896. [PubMed: 19389687]
26. Kohl P, Noble D. Mechanosensitive connective tissue: potential influence on heart rhythm. *Cardiovasc Res* 1996;32:62–68. [PubMed: 8776404]
27. Xie Y, Garfinkel A, Weiss JN, et al. Cardiac Alternans Induced by Fibroblast-Myocyte Coupling: Mechanistic Insights from Computational Models. *Am J Physiol Heart Circ Physiol*. 2009 (in press).
28. Kagiya Y, Hill JL, Gettes LS. Interaction of acidosis and increased extracellular potassium on action potential characteristics and conduction in guinea pig ventricular muscle. *Circ Res* 1982;51:614–623. [PubMed: 7139880]
29. Shaw RM, Rudy Y. Electrophysiologic effects of acute myocardial ischemia: A mechanistic investigation of action potential conduction and conduction failure. *Circ Res* 1997;80:124–138. [PubMed: 8978331]
30. Kohl P, Camelliti P. Cardiac myocyte-nonmyocyte electrotonic coupling: implications for ventricular arrhythmogenesis. *Heart Rhythm* 2007;4:233–235. [PubMed: 17275764]
31. Luo CH, Rudy Y. A model of the ventricular cardiac action potential: depolarization, repolarization, and their interaction. *Circ Res* 1991;68:1501–1526. [PubMed: 1709839]
32. Kohl P, Kamkin AG, Kiseleva IS, et al. Mechanosensitive fibroblasts in the sino-atrial node region of rat heart: interaction with cardiomyocytes and possible role. *Exp Physiol* 1994;79:943–956. [PubMed: 7873162]
33. Vazquez C, Moreno AP, Berbari EJ. Modeling fibroblast-mediated conduction in the ventricle. *Computers Cardiol* 2004;31:349–352.
34. Hyde A, Blondel B, Matter A, et al. Homo- and heterocellular junctions in cell cultures: an electrophysiological and morphological study. *Prog Brain Res* 1969;31:283–311. [PubMed: 4899410]
35. Kohl P. Heterogeneous cell coupling in the heart: an electrophysiological role for fibroblasts. *Circ Res* 2003;93:381–383. [PubMed: 12958139]
36. Kamkin A, Kiseleva I, Wagner KD, et al. Mechanically induced potentials in fibroblasts from human right atrium. *Exp Physiol* 1999;84:347–356. [PubMed: 10226175]
37. Hubbard ML, Ying W, Henriquez CS. Effect of gap junction distribution on impulse propagation in a monolayer of myocytes: a model study. *Europace* 2007;9(Suppl 6):vi20–28. [PubMed: 17959689]
38. Kucera JP, Rudy Y. Mechanistic insights into very slow conduction in branching cardiac tissue: a model study. *Circ Res* 2001;89:799–806. [PubMed: 11679410]
39. Pope AJ, Sands GB, Smaill BH, et al. Three-dimensional transmural organization of perimysial collagen in the heart. *Am J Physiol Heart Circ Physiol* 2008;295:H1243–H1252. [PubMed: 18641274]
40. Qu Z, Karagueuzian HS, Garfinkel A, et al. Effects of Na⁺ channel and cell coupling abnormalities on vulnerability to reentry: a simulation study. *Am J Physiol Heart Circ Physiol* 2004;286:H1310–1321. [PubMed: 14630634]
41. Baudino TA, Carver W, Giles W, et al. Cardiac fibroblasts: friend or foe? *Am J Physiol Heart Circ Physiol* 2006;291:H1015–1026. [PubMed: 16617141]
42. Vazquez C, Feig JE, Mohandas P, et al. Arrhythmogenic potential of activated fibroblasts. *Biophys J* 2009;96:562a–563a.

43. Camelliti P, Devlin GP, Matthews KG, et al. Spatially and temporally distinct expression of fibroblast connexins after sheep ventricular infarction. *Cardiovasc Res* 2004;62:415–425. [PubMed: 15094361]

Figure 1. Effects of Fibroblast-myocyte coupling on CV in random fibroblast attachment model
A. Schematic plot of a 2D tissue model with random fibroblast attachment. **B.** Longitudinal CV (CV_L) versus F-M ratio for different G_f with $E_f = -20$ mV. Arrows indicate F-M ratios at which conduction fails in the tissue. **C.** Dependence of CV_L and resting membrane potential of homogeneous one-dimensional cable of coupled myocytes versus extracellular potassium concentration $[K^+]_o$. **D.** Averaged myocyte resting potential measured from the 2D tissue in B versus F-M ratio for different G_f . Arrows indicate the predicted F-M ratios at which conduction fails, agreeing with the ones in B. **E.** Longitudinal CV (CV_L) versus F-M ratio for different G_f with $E_f = -80$ mV. Tissue size is 2.5×0.5 cm.

Figure 2. Effects of Fibroblast-myocyte coupling on CV in random fibroblast insertion model
A. Schematic plot of a 2D tissue model with random fibroblast insertion. **B.** Longitudinal CV (CV_L) versus F-M ratio at different G_f with $E_f = -20$ mV. **C.** Averaged myocyte resting potential measured from the 2D tissue versus F-M ratio for different G_f . **D.** CV_L versus F-M ratio for different G_j . **E.** CV_L versus G_j for different G_f with an F-M ratio 1:1. Tissue size is 2.5×0.5 cm.

Figure 3. Effects of fibroblast-myocyte coupling on CV in laterally preferential fibroblast-myocyte coupling

A. Confocal microscopy of ventricular myocardium, showing myocytes (red), fibroblasts (blue), and connexin43 (bright green dots), from Camelliti et al.⁸. **B.** Schematic plot of the tissue model of laterally coupled fibroblasts. The small red boxes in the fibroblast rows indicate the transverse myocytes couplings. **C.** Longitudinal CV (CV_L) versus F-M ratio at different G_f . **D.** Transversal CV (CV_T) versus F-M ratio at different G_f . Tissue size is 2.5×0.5 cm.

Figure 4. Effects of Fibroblast-myocyte coupling on CV in laminar sheet structure

A. Left: Transmural image of left ventricular free wall showing laminar layers and clefts, modified from Fig. 2A of Pope et al.³⁹. **Right:** Confocal microscopy tissue cross-section of ventricular myocardium [myocytes (red), fibroblasts (blue), and connexin43 (bright green dots)] from Camelliti et al.⁸, showing the dense network of fibroblasts that surrounds myocyte clusters of 2 to 4 cells. **B.** A schematic plot of a computational model of the laminar sheet structure. The clefts drawn do not necessary equal to the ones we used. **C** and **D.** CV_L and CV_T versus F-M ratio for different G_f . Each data point shows the CV averaged over 16 random structures $\pm$ standard deviation. Tissue size is 2.5×1 cm.

Figure 5. Fibroblasts increase reentry vulnerability in tissue with regional random fibroblast attachment

A. Voltage snapshots showing reentry induced by the premature beat S2 when fibroblasts were attached in the center. **B.** G_j versus the S1S2 interval in which reentry was induced. **C.** G_f versus the S1S2 interval in which reentry was induced. **D.** F-M ratio versus the S1S2 interval in which reentry was induced. Tissue size is 5×1.5 cm and fibroblasts were attached to myocytes in the elliptic region in the tissue center with the long axis of 1.5 cm and short axis of 0.6 cm. F-M ratio is 3:2 in A–C.

Figure 6. Fibroblasts increase reentry vulnerability in tissue with regional random fibroblast insertion

A. Voltage snapshots showing reentry induced by the premature beat when fibroblasts were inserted in the center. **B.** G_j versus the S1S2 interval in which reentry was induced. **C.** G_f versus the S1S2 interval in which reentry was induced. **E.** F-M ratio versus the S1S2 interval in which reentry was induced. The sizes of the tissue, the fibroblast region, and F-M ratios are the same as in Fig. 5.

Figure 7. Fibroblast-myocyte coupling promotes post-repolarization refractoriness which increases the vulnerability to reentry

A. Voltage traces showing determining of ERP. ERP is defined as the minimum S1S2 interval that an S2 can elicit an action potential. The gray traces are that S2 failed to elicit an action potential, while the black ones are action potentials elicited with minimum S1S2 intervals. The stimulus strength is $40 \mu\text{A}/\text{cm}^2$ with 1 ms duration. **B–D.** ΔERP (solid lines) and ΔAPD (dashed lines) measured from a myocyte coupled with fibroblasts, and ΔS1S2 (symbols) from Figs. 5 and 6 versus G_j (B), G_f (C) and F-M ratio (D). ΔS1S2 was defined as the maximum S1S2 interval that reentry was induced less the S1S2 interval for stimulation failure (270 ms in Figs. 5 and 6). ΔERP and ΔAPD were defined as the ERP and APD less the reference values which are ERP and APD at the parameter (G_j , G_f , or F-M ratio) that ΔS1S2 becomes nonzero, so that $\Delta\text{ERP}=0$ and $\Delta\text{APD}=0$ when ΔS1S2 starts to become nonzero.

Figure 8. Fibroblast-myocyte coupling increases the vulnerability to reentry in tissue with laminar structure

A. A schematic plot of a bridge composed of 8 myocytes (see inset) in a single cleft. **B.** Schematic plot of a tissue model with a region (dashed box) in which clefts of random length averaged at 1.25 mm and with 2 to 4 cells between two neighboring clefts. **C and D.** Voltage snapshots during S1S2 stimulation without fibroblasts in the clefts (**C**) and with fibroblasts in the cleft (**D**). S1 was applied at $t=0$ and S2 was applied at $t=270$ ms, both at the lower border of the tissue. In **D**, the F-M ratio in the dashed box region is 0.2, and $G_f=2$ nS.