Cardiac alternans induced by fibroblast-myocyte coupling: mechanistic insights from computational models

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Submitted 8 April 2009; accepted in final form 25 May 2009

The effects of fibroblasts on cardiac electrophysiology have been investigated in experimental studies of cultured monolayers (13, 32, 33, 47, 57) and computational models (19, 20, 29, 40, 41, 45, 57). Gaudesius et al. (13) showed that insertion of fibroblasts into cultured myocyte strands did not block electrical conduction but delayed conduction until a critical length of inserted fibroblasts was reached, demonstrating that myocytes and fibroblasts are electrotonically coupled and that an electrical pulse can conduct through a certain distance in fibroblasts. Both experimental and computational studies (19, 32, 57) have demonstrated that fibroblast-myocyte coupling caused nonmonotonic changes (first an increase and then a decrease) in conduction velocity (CV) as fibroblast content or coupling strength increased. It has also been shown in computational studies (19, 29, 41) that fibroblast-myocyte coupling modulated action potential morphology and action potential duration (APD), depending on the resting potential of the fibroblasts. Fibroblast-myocyte coupling could also depolarize myocyte membrane potential sufficiently to induce automaticity (33).

In this study, we used mathematical modeling and computer simulation to explore the mechanisms by which fibroblast-myocyte coupling affects repolarization and intracellular calcium (Ca2+) alternans dynamics. Our study shows that fibroblast coupling promotes both repolarization and Ca2+ cycling alternans as well as novel alternans dynamics at the cellular and tissue scales. Since repolarization alternans and Ca2+ alternans have been mechanistically linked to cardiac arrhythmogenesis (8, 12, 34, 36, 39), the latter findings may be relevant to the increased arrhythmia risk in heart disease (12, 35, 54), in which structural remodeling promotes increased fibrosis.

METHODS

Fibroblast-myocyte coupling. The membrane voltage (V) of a myocyte (V_m) or a fibroblast (V_f) in coupled myocyte-fibroblast pairs (Fig. 1, A and B) or in tissue (Fig. 1C) is governed by the following:

\[ C \frac{dV}{dt} = -I_{mem} + \sum_{i=1}^{n} G_{gk}^i (V^i - V) \]  

(1)

where C is the membrane capacitance of a myocyte (C_m) or a fibroblast (C_f), t is time, I_{mem} is the corresponding membrane current [of a myocyte (I_m) or a fibroblast (I_f)], n is the number of coupled neighbors (either myocytes or fibroblasts), and \( G_{gk}^i \) is the gap junction conductance between a cell (either a myocyte or a fibroblast) and its kth neighbor (either a myocyte or a fibroblast). The size of the myocyte was set to 125 × 25 × 25 μm, with C_m = 125 pF. For I_m, we used two different action potential models to distinguish between the effects of fibroblast coupling arising purely from membrane ionic currents and those arising from the bidirectional interaction between membrane ionic currents and Ca2+ cycling. For the former model, we used phase I of the Luo and Rudy (LR1) model (28) with modifica-

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action potential properties (including APD alternans) are mediated
by a myocyte and a fibroblast, respectively; in the two-dimensional
tissue model with random fibroblast insertions (hatched squares), which can be
either coupled or uncoupled with myocytes.

Fed and varied from 0.3 to 8 nS in cultured cells, whereas 0
$G_f$ ranged from 0.3 to 8 nS in cultured cells, whereas 0
$G_f$ could be altered independently to explore their
effects on APD and alternans dynamics; 2) the experimentally
measured range of $E_f$ varies from −50 to 0 mV (5, 18, 22, 23, 38), which
are simply passive obstacles.

Fig. 1. Schematic plots of fibroblast-myocyte coupling. A: a myocyte coupled
with one or more fibroblasts. B: an electrical circuit representation of a
myocyte coupled to a fibroblast. $G_m$ and $G_s$, total membrane conductance
of a myocyte and a fibroblast, respectively; $C_{m}$ and $C_s$, membrane
conductance of a myocyte and a fibroblast, respectively; $G_{j}$, gap junction conductance between
a myocyte and a fibroblast; $I_{gap}$, gap junction current. C: two-dimensional
tissue model with random fibroblast insertions (hatched squares), which can be
either coupled or uncoupled with myocytes.

In the two-dimensional (2-D) ventricular tissue
model, fibroblasts were randomly inserted between myocytes, as
described in (15, 56), which is most prominent in the epicardial
region of the heart and is bidirectionally coupled to voltage, as in the realistic
physiological setting. The size of the fibroblast was set to 25 × 25 ×
25 μm. For $I_{f}$, we used a “passive” model (26), as follows:

$$I_{f} = G_f (V - E_f)$$

where $G_f$ is the membrane conductance of a fibroblast and $E_f$ is the
resting potential of a fibroblast. We also carried out simulations using an
“active” model developed by MacCannell et al. (29) for comparison;
these results are described in the Supplemental Material. We
used the passive model as our primary model for the following
reasons: 1) $G_r$ and $E_r$ could be altered independently to explore their
effects on APD and alternans dynamics; 2) the experimentally
measured range of $E_r$ varies from −50 to 0 mV (5, 18, 22, 23, 38), which
may be directly adjusted in the passive, but not the active, model; and 3) as we show in this study, the two models give rise to the same
results qualitatively. In addition, many of the electrophysiological
properties of the fibroblast are still unknown; therefore, the use of a
phenomenological (passive) model is reasonable for qualitative
experiments. In this study, we fixed $C_t = 25$ pF, which is in the
experimentally estimated range of 6.3–75 pF (29, 48). We varied $G_t$
range: 0.1–4 nS based on experimental estimates (26) and $E_t$ to
examine the effects of fibroblasts on the action potential dynamics of
the myocyte.

$Tissue model$. In the two-dimensional (2-D) ventricular tissue
model, fibroblasts were randomly inserted between myocytes, as
shown in Fig. 1C. To generate this tissue model, we first generated a
mesh with a spatial resolution of 25 × 25 μm. A fibroblast accounts
for one such box, whereas a myocyte accounts for five boxes in a row.
Once a fibroblast-myocyte ratio (α) is assigned, then the probability of

1 Supplemental material for this article is available at the American Journal
of Physiology-Heart and Circulatory Physiology website.

Fibroblast-myocyte coupling gives rise to an early transient
outward current and a late background current for the myocyte.
When either the LR1 model or the rabbit ventricular
model is coupled to a fibroblast, the resting potential of the
fibroblast becomes markedly hyperpolarized and that of
the myocyte slightly elevated. The extent of these alterations
depends on the membrane conductance of the myocyte, $G_m$ and
$G_f$ (see Supplemental Material), as also shown in a previous
study by Jacquemet and Henriquez (19). For example, when
we coupled the LR1 model to a fibroblast with a low $G_f$, the
resting potential of the fibroblast was only slightly higher than
that of the myocyte (Fig. 2A). After a stimulus was applied to
the myocyte, its voltage increased from around −80 mV to
above 0 mV in <1 ms. However, since the inexcitable fibro-
blast is equivalent to a leaky capacitor, it took several milli-
seconds for the myocyte to charge the fibroblast and elevate its
voltage from −80 mV to close to 0 mV. Due to the time delay,
the initial voltage difference between the myocyte and fibro-
blast was very large, giving rise to a large gap junction current
($I_{gap}$), which flowed from the myocyte to the fibroblast (Fig.
2B). After that, the voltage of the fibroblasts was close to that
of the myocyte, and $I_{gap}$ became small. Whether this late
component of $I_{gap}$ flows from the myocyte to the fibroblast or
vice versa depends on the properties of the fibroblast. Since
the early phase of $I_{gap}$ is a short pulse and outward for the myocyte,
It resembles the fast transient outward current ($I_{to}$) in cardiac
myocytes (15, 56), which is most prominent in the epicardial
layer (27, 56). Figure 2, C and D, shows $I_{f}$ for $G_f = 0.1$ and 4
nS, which are inward currents during the diastolic phase but
outward during the systolic phase. Thus, rather than a pure
passive load or driver, the fibroblast acts like a leaky capacitor,
charging during the resting potential and upstroke, and then

$V_{APD}$ was defined as the tissue length divided by the time for the action
potential to conduct from the left end to the right end of the tissue.
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leaking current throughout the action potential plateau of the myocyte.

Using a voltage-clamp model of the myocyte, we can analytically derive the decay time of the pulse [time constant (τ)], which is \( \tau = C_0(\text{G}_f + C_f) \) (see Supplemental Fig. S1 and the Supplemental Material). The peak of \( I_{\text{gap}} \) is insensitive to \( \text{G}_f \) and increases with \( \text{G}_f \) almost linearly unless \( \text{E}_f \) is high (Fig. 2E). In the late phase, \( I_{\text{gap}} \) can be approximated as follows (see the derivation of Eq. S27 in the Supplemental Material):

\[
I_{\text{gap}} = \frac{\text{G}_f \text{C}_f}{\text{G}_f + \text{C}_f} (V_m - V_c)
\]

where \( V_c \) is the voltage at which \( V_m \) crosses \( V_f \), defining the point at which \( I_{\text{gap}} \) changes from an outward current (tending to shorten APD) to an inward current (tending to lengthen APD). 

**Equation 3** agrees well with the simulation results of the LR1 and rabbit ventricular models coupled to the passive fibroblast model (Fig. 2F). Similar linear relations occurred when the LR1 and rabbit ventricular models were coupled to the active fibroblast model (Supplemental Fig. S1). Note that in the repolarizing phase, \( I_{\text{gap}} \) is proportional to both \( \text{G}_f \) and \( \text{G}_i \).

These two characteristics of \( I_{\text{gap}} \) give rise to different and nontrivial action potential dynamics. In the following sections, we show how the fibroblast-mycyte coupling affects APD, APD alternans, and Ca\(^{2+}\) alternans at both the cellular and tissue scales.

**Fibroblast-mycyte coupling alters APD**. Previous modeling studies (19, 29, 41) have shown that fibroblast-mycyte coupling can either prolong or shorten APD, depending on the specific ionic models of myocytes and fibroblasts, their membrane and coupling parameters, and also on the threshold used to define APD. However, the general mechanisms by which APD is affected by fibroblast-mycyte coupling are not clear.

Here, we used the APD at 90% repolarization (see METHODS for the definition) and scanned the \( \text{E}_f \) from \(-80 \) to 0 mV and \( \text{G}_i \) from 0 to 4 nS to show how APD is altered in a coupled cell pair of a passive fibroblast and the LR1 myocyte model. APD was shortened only when \( \text{E}_f \) was low and \( \text{G}_f \) was high (Fig. 3A). Note that APD was still prolonged even for an (unphysiological) value of \( \text{E}_f = -80 \) mV, close to the resting potential of the myocyte.

Based on the properties of \( I_{\text{gap}} \), we can understand the general mechanisms by which fibroblast-mycyte coupling affects APD as follows. When \( \text{G}_i \) is small, \( I_{\text{gap}} \) in the repolarizing phase is small and becomes inward at a \( V_c \), regardless of \( \text{E}_f \), tending to prolong APD. The early phase of \( I_{\text{gap}} \) is similar to the fast \( I_{\text{lo}} \), which lowers the action potential notch and prolongs APD in the LR1 model, as also shown in other

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**Fig. 2. Effects of fibroblast-mycyte coupling on action potential duration (APD) in the phase I Luo-Rudy (LR1) model.** A: Action potential waveform from a myocyte before (shaded line) and after (solid line) coupling to a fibroblast in a myocyte-fibroblast pair. The membrane voltage (\( V \)) of the coupled fibroblast (\( V_f \)) during the action potential is also shown (dashed line). \( V_c \) is the critical voltage at which \( V_f \) crosses the membrane voltage of a myocyte (\( V_m \)). Pacing cycle length (PCL) = 500 ms, \( E_f = -50 \) mV, \( \text{G}_i = 0.1 \) nS, and \( \text{G}_f = 3 \) nS were used. \( t, \text{time} \). B: \( I_{\text{gap}} = G_f(V_m - V_c) \), which is injected by the fibroblast into the myocyte, for \( \text{G}_i = 0.1 \) (shaded line) and 4 nS (solid line), illustrating the early transient outward current (\( I_{\text{lo}} \)) component followed by a smaller sustained component. The relative amplitudes of the two components varies with \( \text{G}_f \). C: Membrane current of a fibroblast (\( I_f \)) versus \( t \) for \( \text{G}_i = 0.1 \) nS. D: \( I_f \) versus \( t \) for \( \text{G}_i = 4 \) nS. E: Dependence of peak \( I_{\text{gap}} \) (i.e., the early \( I_{\text{lo}} \) component) on \( \text{G}_i \), with \( \text{G}_f = 0.1 \) (open symbols) and 4 nS (solid symbols), with \( E_f = 50 \) (squares) or \(-20 \) mV (circles), using the LR1 model coupled to the passive fibroblast model. PCL = 500 ms. F: Dependence of \( I_{\text{gap}} \) during the repolarizing phase of the action potential (i.e., the late component) on \( V_m \) using the LR1 model (solid trace) or rabbit ventricular model (shaded trace) coupled with the passive fibroblast model. The dashed line is a plot of Eq. 3 with \( V_c = -48.5 \) mV and showed good agreement. PCL = 500 ms, \( E_f = -50 \) mV, \( \text{G}_i = 4 \) nS, and \( \text{G}_f = 3 \) nS were used.
models (15). Therefore, APD always prolongs when $G_f$ is small irrespective of $E_f$. When $G_f$ is large, however, the late phase of $I_{\text{gap}}$ is large and exerts a major effect on APD. Since $V_c \sim E_f$ for large $G_f$, then if $E_f$ is high, this current is mainly inward and lengthens APD; however, if $E_f$ is low, this current is mainly outward and thus shortens APD. This explains why APD only shortens when $G_f$ is large and $E_f$ is low (see Fig. 3A, top left corner).

When the rabbit ventricular model was coupled to the passive fibroblast model, we observed exactly the same behaviors as in the LR1 model (Supplemental Fig. S2). Similar results were also obtained when the LR1 model or rabbit ventricular model was coupled to the active fibroblast model formulated by MacCannell et al. (29): APD shortened when the resting potential of the fibroblast was low but prolonged when the resting potential was high, and $I_{\text{gap}}$ was similar to that of using the passive fibroblast model (Supplemental Fig. S3).

**Fibroblast-myocyte coupling modulates voltage-driven APD alternans.** Since fibroblast-myocyte coupling alters APD, it will also change the critical pacing cycle length (PCL) at which alternans occurs. For small $G_f$, APD prolongs and APD alternans occurs at a longer PCL, whereas for large $G_f$, APD shortens and APD alternans occurs at a shorter PCL (Fig. 3B). We scanned $E_f$ from $-80$ to $0$ mV and $G_f$ from $0$ to $4$ nS for the difference in critical PCL ($\Delta\text{PCL}_{c}$) from that of the control myocyte (Fig. 3C), which was similar to the pattern of $\Delta\text{APD}$ in Fig. 3A. Note that the change in PCLc was much larger than the change in APD ($\Delta\text{PCL}_{c}$ ranged from $-107$ to $+60$ ms in Fig. 3C, whereas $\Delta\text{APD}$ ranged from $-45$ to $+22$ ms in Fig. 3A). Therefore, the change in the alternans onset is due not just to the prolongation or shortening of APD. To understand this difference, we plot APD restitution curves for the control, small, and large $G_f$ in Fig. 3D and the corresponding slopes in Fig. 3E. When APD was prolonged, the APD restitution curve became steeper, and when APD was shortened, the APD restitution curve became less steep. APD alternans is due to the steep slope of the APD restitution curve in this model, which explains why the change in the alternans onset PCL is much larger than the change in APD. The same effects were observed when the active fibroblast model was coupled to the LR1 model (Supplemental Fig. S4).

**Fibroblast-myocyte coupling results in novel APD alternans dynamics.** In addition to altering the onset of APD alternans during pacing at fast heart rates, fibroblast-myocyte coupling causes a new dynamical instability that leads to alternans at slow heart rates. For the small $G_f$ case, when the number of fibroblasts attached to a myocyte was increased (equivalent to scaling up $C_t$, $G_f$, and $G_i$ for a single virtual fibroblast), the notch of the action potential became progressively lower, and APD first prolonged and then shortened as the number of attached fibroblasts increased (Fig. 4, A and B). The curve relating APD to the number of attached fibroblast was not smooth but shortened suddenly at two critical points (Fig. 4B). These features are similar to fast $I_{\text{on}}$, which first increases APD and then shortens APD as its conductance increases (10, 15, 17), ultimately causing “all-or-none” repolarization. The mechanism (15) is that $I_{\text{on}}$ first lowers the action potential notch, increasing the driving force for inward current through L-type

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**Fig. 3.** Effects of fibroblast-myocyte coupling on APD and voltage-driven alternans in the LR1 model. A: change in APD ($\Delta\text{APD} = \text{APD} - \text{APD}_{\text{control}}$) when the myocyte is coupled to two passive fibroblasts in $G_f$-$E_f$ parameter space, where $\text{APD}_{\text{control}}$ is the APD of the control uncoupled myocyte. PCL = 500 ms and $G_f = 8$ nS were used. B: APD versus PCL for a control myocyte versus a myocyte coupled to two passive fibroblasts of $G_f = 0.1$ and 4 nS. APD is a single value for each PCL before alternans occurs but two APD values (long and short) after alternans occurs. C: change in critical PCL ($\Delta\text{PCL}_{c} = \text{PCL}_{c} - \text{PCL}_{c,\text{control}}$) distribution in $G_f$-$E_f$ parameter space, where $\text{PCL}_{c}$ is the PCL at which alternans occurs in the myocyte coupled to fibroblasts and $\text{PCL}_{c,\text{control}}$ is the PCL at which alternans occurs in the control myocyte. D: APD versus diastolic interval (DI) obtained by the S1S2 protocol from a control myocyte (solid line) versus a myocyte coupled to two passive fibroblasts (red line) and two active fibroblasts (blue line). In both cases, $E_f = -50$ mV and $G_f = 3$ nS. E: APD restitution slope versus DI for the curves in D. Two passive fibroblasts were coupled to the myocyte.
calcium channels and delaying the activation of time-dependent potassium currents, thus lengthening the APD. As $I_{to}$ becomes larger, however, the early repolarization phase interferes with the full activation of the L-type calcium current ($I_{Ca,L}$), leading to action potential shortening and, with further increases, to all-or-none repolarization. Since for small $G_f$ the dominant component of $I_{gap}$ is the early $I_{to}$-like phase, it is not surprising that coupling more fibroblasts to the myocyte has the same effect as increasing the conductance of $I_{to}$. The effects of increasing the number of attached fibroblasts on the onset of APD alternans are shown in Fig. 4C. PCLc increased dramatically and then suddenly dropped as the number of attached fibroblasts increased. In addition to alternans at short PCLs (~200 ms), the bifurcation diagram shown in Fig. 4D (see also the representative voltage trace at PCL = 500 ms in Fig. 4E) demonstrates that a new APD alternans region occurs at long PCLs. Unlike APD alternans at short PCLs, which in the LR1 model is due to the slope of the APD restitution exceeding 1 (Fig. 3), the slope of APD restitution is <1 during APD alternans at long PCL. Since $I_{gap}$ is similar to a fast $I_{to}$, the mechanism of alternans is likely to be the same as demonstrated in a recent modeling study by Hopenfeld (17), who showed that the interplay between $I_{Ca,L}$ and $I_{to}$ can give rise to a novel voltage-driven mechanism of APD alternans and complex APD dynamics. This same effect of $I_{to}$ on alternans dynamics at slow heart rates has been shown in experiments by Lukas and Antzelevitch in canine hearts during ischemia (27).

**Fibroblast-myocyte coupling promotes $Ca_i$ alternans.** In the above simulations, we used the LR1 model to characterize the direct influence of $I_{gap}$ on membrane ionic currents during the action potential in the absence of complicating effects of $Ca_i$, since this model does not contain detailed $Ca_i$ cycling. In real myocytes, however, $Ca_i$ cycling is a major factor driving APD alternans (14, 51). To investigate how the addition of $Ca_i$ cycling affects alternans, we used the rabbit ventricular model in place of the LR1 model. When fibroblasts were coupled to the rabbit ventricular model, the effects of $I_{gap}$ on action potential morphology were generally similar to those in the LR1 model (Supplemental Fig. S2). Differing from the LR1 model, however, fibroblast coupling promoted alternans whether the APD was prolonged (small $G_f$) or shortened (large $G_f$) (Fig. 5). This is not completely surprising, since in the rabbit ventricular model, the onset of alternans is primarily driven by a $Ca_i$ cycling instability rather than APD restitution slope (30). Under control conditions in the rabbit ventricular model, alternans occurred at PCLc = 250 ms (Fig. 5A) and was electromechanically concordant, i.e., a long APD was associated with a large $Ca_i$ transient, and vice versa. When coupled to two fibroblasts of $G_f = 1.2$ nS, alternans occurred at a longer PCLc of 305 ms (Fig. 5B) and remained electromechanically concordant. When coupled with two fibroblasts of $G_f = 0.1$ nS, however, alternans occurred at PCLc = 305 ms and was electromechanically discordant, i.e., a long APD is associated with a small $Ca_i$ transient, and vice versa (Fig. 5C).
alternans remained discordant until PCL = 272 ms and then became concordant.

Since $I_{to}$ augments the Ca transient amplitude by increasing the driving force through $I_{to,L}$ during the early action potential (16, 30, 42, 43), we examined whether $I_{to}$-like early phase of $I_{gap}$ accounted for the promotion of alternans. To increase the amplitude of the early $I_{to}$-like phase of $I_{gap}$, we increased $G_j$ and then examined how this affected PCLc (Fig. 5D). As $G_j$ was increased, PCLc for the onset of alternans first increased from 250 to ~400 ms and then decreased for $G_j = 0.1$ nS. If $G_f$ was larger (1.2 nS), however, PCLc increased and saturated, without a decrease. When the conductance of the fast $I_{to}$ ($G_{to,f}$) in the rabbit ventricular model ($G_{to,f} = 0.11$ mS/cm² in the original model) was increased in the absence of fibroblast coupling, the PCLc for alternans increased (Fig. 5E). When we fixed $G_{to,f} = 0.15$ mS/cm² and plotted APD and peak Ca in the rabbit ventricular model uncoupled to fibroblasts. Therefore, by comparing the two sets of simulation studies, we conclude that the $I_{to}$-like effect of the fibroblast-myocyte coupling may cause the promotion of Ca alternans. However, in the case of fibroblast-myocyte coupling, increasing $G_f$ also increases the late phase of $I_{gap}$, which may account for the difference between the two sets of simulations. Although we have not pinpointed the exact mechanism that $I_{to}$ or the $I_{to}$-like effect due to fibroblast-myocyte coupling promotes Ca alternans, Jordan and Christini (21) have shown that action potential morphology modulates the onset of Ca alternans, which may explain this effect.

Fibroblast-myocyte promotes spatially discordant alternans in tissue. In cardiac tissue, APD alternans can be spatially concordant or discordant (34, 36), with the latter being more arrhythmogenic (34). Since CV restitution (the dependence of CV on heart rate) has been shown to be important in the development of spatially discordant alternans (36), we examined the effects of fibroblast-myocyte coupling on CV restitution. In homogenous 2-D tissue using the LR1 model, as shown in Fig. 1B, CV was ~0.56 m/s without fibroblasts. When fibroblasts were randomly inserted at a 1:1 ratio with myocytes, the CV longitudinal to fiber direction decreased to ~0.33 m/s for either coupled or uncoupled fibroblasts, whereas CV in the transverse direction decreased from 0.13 to 0.1 m/s. CV started to decrease at longer PCLs when fibroblasts were coupled with the myocytes than when fibroblasts were uncoupled or absent (Fig. 6A), indicating that fibroblast-myocyte coupling broadens the PCL range over which CV changes. Under control conditions without fibroblasts (Fig. 6B, solid squares) or with randomly inserted uncoupled fibroblasts (Fig. 6B, open squares), the PCLc at the onset of alternans was similar. When the fibroblasts (with a small $G_f$) were coupled, however, alternans occurred at a longer PCLc, as in the single nodal cell case. In the 2-D tissue, without fibroblasts, a single nodal

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**Fig. 5. Effects of fibroblast-myocyte coupling on calcium-driven alternans in the rabbit ventricular model.** A–C: bifurcation diagrams showing APD (solid circles) and peak intracellular calcium (Ca; open circles) versus PCL from a control myocyte uncoupled to fibroblasts (A) and a myocyte coupled to three fibroblasts with $G_f = 1.2$ (B) and 0.1 nS (C). D: PCLc of a myocyte coupled to three fibroblasts versus $G_j$ for $G_f = 0.1$ (solid circles) and 1.2 nS (open circles). E: PCLc versus the conductance ($G_{to,f}$) in the rabbit ventricular model uncoupled to fibroblasts. The arrow indicates the $G_{to,f}$ value (~0.11 mS/cm²) in the original rabbit ventricular model. F: bifurcation diagram of APD (solid circles) and peak Ca (open circles) for $G_{to,f} = 0.15$ mS/cm² in the rabbit ventricular model uncoupled to fibroblasts.
line formed beyond the middle of the tissue when PCL = 280 ms (Fig. 6C). With uncoupled fibroblasts inserted at a 1:1 fibroblast-to-myocyte ratio, a single nodal line formed closer to the pacing site (Fig. 6D), consistent with the principle that slowing CV effectively increases the tissue size. With fibroblasts coupled to myocytes, however, two nodal lines formed in the tissue and the amplitude of alternans was increased (Fig. 6E). Thus, fibroblast-myocyte coupling promotes spatially discordant APD alternans by slowing CV and broadening CV restitution.

To examine how Ca cycling and electromechanical concordance/discordance affects spatially discordant alternans, we simulated 2-D tissue using the rabbit ventricular model, with randomly inserted passive fibroblasts with small \( G_f \) (0.1 nS) at a fibroblast-to-myocyte ratio of 2:1. At a PCL of 200 ms, for which alternans was electromechanically discordant (Fig. 5C), spatially discordant APD and Ca alternans formed and electromechanical discordance was maintained (Fig. 7A). The formation of spatially discordant alternans in this case was due to the engagement of CV restitution. In the same tissue at a longer PCL of 290 ms, in which CV restitution was not yet engaged, APD alternans remained spatially concordant but was electromechanically discordant (Fig. 7B). An interesting case occurred at an intermediate PCL of 260 ms, in which the fibroblast-to-myocyte ratio was increased linearly from 1:9 to 2:1 from left to right. This produced electromechanically concordant alternans at both the left and right sides but electromechanically discordant alternans at the middle, resulting in spatially discordant APD alternans yet spatially concordant Ca alternans (Fig. 7C). This is a novel mechanism for the formation of spatially discordant alternans, consistent with the observations of Wilson et al. (53, 54), who showed that regional differences in electromechanical concordance and discordance in heart failure led to spatially discordant alternans.

**DISCUSSION**

In this study, we analyzed the mechanisms by which fibroblast-myocyte coupling modulates action potential morphol-
The passive fibroblast model was used with 290 ms, with a fibroblast-to-myocyte ratio of 2:1, which is a common factor in many forms of heart disease. Cyto coupling amplifies the proarrhythmic effects of fibrosis, revealing generic mechanistic insights into how fibroblast-myo... analysis and findings, obtained using several different models, specific fibroblast membrane properties and coupling parameters, a richly complex interaction results. The mathematical... decay time of the fast transient component due to the current leak through the fibroblast’s membrane capacitance, followed... amplitude is proportional to the leakiness of the capacitor (equivalent to a nonselective background current whose gap is large and the late phase is small, the effects of fibroblast coupling on action potential dynamics are very similar to those of \( I_{\text{to}} \), as we have shown by direct comparison. In contrast, when \( G_f \) is low (e.g., \(-50 \text{ mV}\)), the current during the later phase of repolarization becomes inward sooner and prolongs APD. The effects of fibroblast-myo... coupling on APD restitution parallel to the effects on APD, with APD restitution becoming less steep when APD is shortened and steeper when APD is prolonged. These effects, in turn, cause the onset of restitution-driven APD alternans to occur at a shorter PCL when APD is shorter and at a longer PCL when APD is prolonged, although these effects are more pronounced than can be explained solely by the degree of change in APD. Due to the fast transient component of \( I_{\text{gap}} \), a novel type of APD alternans that occurs at slow heart rates is observed, similar to the alternans caused by \( I_{\text{to}} \) shown in experimental (27) and modeling (17) studies.

In addition to the effects on voltage-driven APD alternans, we also found that fibroblast-myo... coupling, the effects of fibroblast coupling on action potential dynamics are very similar to those of \( I_{\text{to}} \), as we have shown by direct comparison. In contrast, when \( G_f \) is large, the second, later phase of \( I_{\text{gap}} \) (equivalent to the capacitor becoming more leaky and unable to hold charge) becomes important. If \( E_f \) is low (e.g., \(-50 \text{ mV}\)), the current is mainly outward and shortens APD. If \( E_f \) is high (e.g., \(-10 \text{ mV}\)), the current during the later phase of repolarization becomes inward sooner and prolongs APD.

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replicated in the rabbit ventricular action potential model (30). Presumably, \( C_{\text{Ca}} \) alternans potentiation by \( I_{\text{Ca}} \) is related to this effect. Similarly, the ability of \( I_{\text{gap}} \) to induce electromechanically discordant alternans was also reproduced by increasing \( I_{\text{Ca}} \). Electromechanically discordant alternans has been experimentally observed in heart failure (53), in which \( I_{\text{Ca}} \) is typically downregulated, so that fibroblast-myocyte coupling may provide an alternative explanation.

**Fibroblasts promote the dispersion of refractoriness in cardiac tissue.** Because fibroblast proliferation and fibrosis in the diseased heart are inherently heterogeneous, the local effects of fibroblast-myocyte coupling on APD and APD restitution will promote increased dispersion of refractoriness. In addition, dispersion of refractoriness can be dynamically generated even in homogeneous tissue by spatially discordant APD and \( C_{\text{Ca}} \) alternans, which we found is also promoted by myocyte-fibroblast coupling through at least two mechanisms. First, fibroblasts broaden the heart rate range over which CV changes, a well-known factor promoting spatially discordant alternans (36). Second, as shown in Fig. 7C, spatial heterogeneity in the fibroblast density can cause alternans to shift from electromechanically concordant to electromechanically discordant alternans over space. A novel mechanism of spatially discordant alternans arising from regional electromechanical concordance/discordance may provide an explanation for the experimental observations of Wilson et al. (53) in an animal model of heart failure, without needing to postulate that primary myocyte remodeling caused the observed regional differences in electromechanical concordance/discordance. Rather, the regional fibroblast density may have been the critical factor.

**Limitations.** Several limitations of this study should be mentioned. Although different fibroblast models have been developed (20, 29, 41), there is limited direct information on fibroblast electrophysiology, such that many elements in the existing models have been formulated phenomenologically. On the other hand, by using multiple models, we demonstrated that the effects of fibroblast-myocyte coupling on APD and alternans dynamics identified here are generic rather than model specific, leading us to believe that the findings are relevant to real cardiac tissue. Regarding the myocyte action potential models, we considered exploring the pure voltage-mediated effects of fibroblast-myocyte coupling using the rabbit ventricular action potential model with its \( C_{\text{Ca}} \) buffered for comparison with the normal (\( C_{\text{Ca}} \) unbuffered) condition. However, \( C_{\text{Ca}} \) buffering with EGTA or BAPTA in rabbit ventricular myocytes has been reported to flatten APD restitution and suppress APD alternans (14), making this option untenable. Therefore, we used the LR1 model as an alternative, despite its simplicity. However, the generally similar effects of fibroblast coupling in both models strengthens the argument that the findings are not likely to be highly model dependent. Another limitation involves the tissue models. As shown by Kohl and Camelli (24), there are at least three different types of fibroblast-myocyte coupling in cardiac tissue (zero-, single- and double-sided connections), and the number and size of attached fibroblasts are likely to be highly variable, which may form heterogeneous patterns of fibroblast distribution. In addition, where fibroblasts are not excitable, they do respond to various neurohumoral factors and manifest their own \( C_{\text{Ca}} \) cycling properties. We did not consider these factors here because information about their electrophysiological responses is limited. Finally, the most controversial aspect relates to whether fibroblasts form functional gap junctions with myocytes in intact cardiac tissue, as they do in fibroblast-myocyte cocultures. In normal hearts, fibroblasts comprise the majority of nonmyocyte cells, accounting for up to two-thirds of all cells in the heart (2, 5). However, direct experimental evidence on fibroblast-myocyte coupling is scarce in the intact working myocardium, having only been demonstrated in the sinoatrial node (7). However, it has been speculated that pathophysiological conditions may promote coupling (50), which is indirectly supported by a recent study (47) showing that cardiac fibroblasts isolated from ischemic hearts expressed significantly more connexin40 and connexin43 than those isolated from normal hearts.

In summary, despite some limitations, our present study provides novel mechanistic insights into how the early and late phases of \( I_{\text{gap}} \) generated by fibroblast-myocyte coupling affect cardiac action potential dynamics in a complex manner and thereby contributes to our understanding of the mechanisms by which fibroblasts may be proarrhythmic in the diseased heart.

**GRANTS**

This work is supported by National Heart, Lung, and Blood Institute Grant P01-HL-078931 and Laubisch and Kawata Endowments.

**REFERENCES**
