Spiral-Wave Dynamics in a Mathematical Model of Human Ventricular Tissue with Myocytes and Fibroblasts

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Abstract

Cardiac fibroblasts, when coupled functionally with myocytes, can modulate the electrophysiological properties of cardiac tissue. We present systematic numerical studies of such modulation of electrophysiological properties in mathematical models for (a) single myocyte-fibroblast (MF) units and (b) two-dimensional (2D) arrays of such units; our models build on earlier ones and allow for zero-, one-, and two-sided MF couplings. Our studies of MF units elucidate the dependence of the action-potential (AP) morphology on parameters such as \( E_f \), the fibroblast resting-membrane potential, the fibroblast conductance \( G_f \) and the MF gap-junctional coupling \( G_{gap} \). Furthermore, we find that our MF composite can show autorhythmic and oscillatory behaviors in addition to an excitable response. Our 2D studies use (a) both homogeneous and inhomogeneous distributions of fibroblasts, (b) various ranges for parameters such as \( G_{gap}, G_f \), and \( E_f \) and (c) intercellular couplings that can be zero-sided, one-sided, and two-sided connections of fibroblasts with myocytes. We show, in particular, that the plane-wave conduction velocity \( CV \) decreases as a function of \( G_{gap} \) for zero-sided and one-sided couplings; however, for two-sided coupling, \( CV \) decreases initially and then increases as a function of \( G_{gap} \), and, eventually, we observe that conduction failure occurs for low values of \( G_{gap} \). In our homogeneous studies, we find that the rotation speed and stability of a spiral wave can be controlled either by controlling \( G_{gap} \) or \( E_f \). Our studies with fibroblast inhomogeneities show that a spiral wave can get anchored to a local fibroblast inhomogeneity. We also study the efficacy of a low-amplitude control scheme, which has been suggested for the control of spiral-wave turbulence in mathematical models for cardiac tissue, in our MF model both with and without heterogeneities.

Introduction

Cardiac fibroblasts, which are connective, non-myocyte cells, play a major role in producing myocyte cells, both in the early stage of heart development and after a myocardial infarction. Experimental studies [1,2] suggest that such fibroblasts can be coupled functionally with myocytes, under both physiological and pathophysiological conditions. Fibroblasts can, therefore, modulate the electrophysiological properties of cardiac tissue. However, it is not clear yet what range of values we should use for the gap-junctional conductance \( G_{gap} \) of a fibroblast-myocyte gap junction [3–5]; in intact tissue \( 4nS \leq G_{gap} \leq 6nS \) [3] and, in cell-culture preparations, \( 0.3nS \leq G_{gap} \leq 8.0nS \) [5]. The structural organization of fibroblast cells in cardiac tissue, which consists of myocyte and non-myocyte cells (e.g., fibroblasts), is still being explored [2,6–8] for different mammalian hearts. This lack of detailed structural and functional information makes it difficult to use experimental studies to uncover the precise role that fibroblasts play in the propagation of electrical impulses and spiral waves of electrical activation in cardiac tissue. Therefore, computational studies are beginning to play an important role in the investigation of the properties of mathematical models for cardiac tissue that include myocytes and fibroblasts and a coupling between them; some of these study a single, composite myocyte-fibroblast cell [9–12]; others have considered electrical-wave propagation in one- and two-dimensional, mathematical models for cardiac tissue, in which the fibroblasts are modelled as passive cells [11,13,14]. Here, we build on mathematical models that couple cardiac myocytes and fibroblasts at the single-cell level (this yields a myocyte-fibroblast MF composite) to develop a mathematical model for a two-dimensional (2D) sheet of cardiac myocytes coupled to a similar sheet of fibroblasts. Our model uses the state-of-the-art ionic model for human cardiac myocytes due to ten Tusscher, Noble, Noble, and Panfilov (TNNP) [15]; we include connections between myocytes and fibroblasts via gap junctions; and we also allow for the possibility of studying zero-sided, one-sided, and two-sided couplings (see section on “Model and Methods”).
We begin with a summary of the principal results of our extensive numerical studies, which we have designed to elucidate electrophysiological properties in mathematical models for (a) single myocyte-fibroblast (MF) units and (b) two-dimensional (2D) arrays of such units. Our studies of MF units yield the dependence of the action-potential (AP) morphology on parameters such as $E_f$, the fibroblast resting-membrane potential, the fibroblast conductance $G_f$, the number $N_f$ of fibroblasts coupled to a myocyte, and the MF gap-junctional coupling $G_{gap}$; and our MF composite can show autorhythmic and oscillatory behaviors in addition to an excitatory response. We also present ionic mechanisms that are responsible for the modulation of the AP as we alter $E_f$ or $G_{gap}$. Our 2D studies use (a) both homogeneous and inhomogeneous distributions of fibroblasts, (b) various ranges for parameters such as $G_{gap}, G_f$, and $E_f$, and (c) zero-, one-, and two-sided MF connections. We show that the plane-wave conduction velocity $CV$ decreases as a function of $G_{gap}$, for zero- and one-sided couplings; however, for two-sided coupling, $CV$ decreases initially and then increases as a function of $G_{gap}$, and, eventually, we observe that conduction failure occurs for low values of $G_{gap}$. In our homogeneous studies, we find that the rotation speed and stability of a spiral wave can be controlled either by controlling $G_{gap}$ or $E_f$. And we show that a spiral wave can get anchored to a local fibroblast inhomogeneity. We demonstrate the efficacy of a low-amplitude control scheme, which has been suggested for the control of spiral-wave turbulence in mathematical models for cardiac tissue [16–19], in our MF model both with and without heterogeneities. We include several animations of our simulations (Videos S1–S12) to provide a quick, pictorial overview of our results.

We now present a brief and illustrative overview of some earlier studies that have investigated the AP morphological behavior at the level of MF units to determine the effects of the extra electrical load, either because of passive or active fibroblasts, in both animal- and human-ventricular-cell models; we also discuss the propagation of electrical impulses in cardiac tissue models with fibroblasts. In Table 1 we give the ranges of parameters that have been used, in a variety of experimental and computational studies, of MF composites.

Xie, et al. [9] have used two different ionic models for myocytes, namely, the Luo-Rudy Phase 1 (LRI) model [20], with modified maximal conductances, and a rabbit-ventricular-cell model [21], coupled to models of passive and active fibroblasts via a gap-junctional conductance (Table 1). In their passive-fibroblast studies, for low values of $G_f$, they have found that the action-potential duration (APD) is always prolonged relative to its value APD$^m$ for an uncoupled myocyte; however, if $G_f$ is large, then the APD is less than APD$^m$, if $E_f$ is low, but greater than APD$^m$, if $E_f$ is high. They have obtained similar results in models with active fibroblasts.

Sachse, et al. [10] have shown, with a rat-ventricular-cell model [22], that the APD is prolonged relative to APD$^m$ in an active-fibroblast model. Their study shows that the myocyte APD, measured at 90% repolarization, increases from 38.9 ms to 61.3 ms if $N_f=10$ and $G_{gap}=10$ nS; however, if $N_f=10$ and $G_{gap}=0.1$ nS, this APD decreases from 39.0 ms to 37.1 ms. Their studies also show that the myocyte resting membrane potential, $V_{rest}$, and the maximal upstroke velocity, $V_{max}$, depend on $N_f$ and $G_{gap}$.

Jacquemet, et al. [11] have studied a mouse-ventricular-cell model [23] coupled to a simple fibroblast model that includes a delayed activation of the membrane current. Their study has revealed that the myocyte APD is prolonged from 14.4 ms to 14.8 ms, its action potential amplitude (APA) reduced from 115.1 mV to 114.6 mV, and there is a slight elevation of the resting membrane potential $V_{rest}$ from ~62.3 mV to ~82.0 mV, when a single fibroblast is coupled to a myocyte with $G_{gap}=4.05$ nS. They have also studied the dependence of the APA, APD, $V_{rest}$, and $V_{max}$ on $N_f$ and $G_{gap}$ by measuring them at a site of a myocyte cell, located in the middle of a cable, which contains 30 myocyte cells covered by a layer of fibroblasts. They have found, e.g., that (a) the APA, APD, and $V_{rest}$ change to 93.1 mV, 19.3 ms, and ~80.5 mV, respectively, from their corresponding uncoupled values 100.8 mV, 15.7 ms, and ~82.3 mV, when $N_f=10$ and $G_{gap}=4.05$ nS, and (b) $V_{max}$ changes to ~68 mV/ms from its uncoupled value ~92 mV/ms, when $N_f=17$ and $G_{gap}=4.05$ nS.

MacCannell, et al. [12] have considered fibroblast models, principally active but also passive, coupled to a human-ventricular-myocyte model [15]. They have presented representative results for a single MF unit for the passive case; they have found that the myocyte APD increases from its uncoupled value 263 ms to 273 ms or 275 ms for $N_f=2$ and 4, respectively. By contrast, in their active-MF model, they have found that the APD decreases from 263 ms to 195 ms and 155 ms, respectively, when $N_f=2$ or 4 (with the above-mentioned parameter values); furthermore, $V_{rest}$ is elevated from its uncoupled value ~86.1 mV to ~85.8 mV, if $N_f=2$, and ~85.3 mV, if $N_f=4$, and the APD shortening can be enhanced by increasing either $C_f$ or $G_{gap}$; e.g., if $N_f=2$ the APD decreases from 263 ms to 225 ms or 207 ms, respectively, for $G_{gap}=1$ and 2 nS, with $C_f=6.3$ pF, $G_f=0.1$ nS, and $E_f=−49.6$ mV. This study also obtains similar results when it holds all parameters at the values given above but uses $C_f=6$ pF or 63 pF.

Both in cell culture and in intact tissue, fibroblasts can couple functionally to adjacent myocytes via a gap junction at the single-cell level by expressing either the Cx43 or the Cx45 gap-junction protein or connexin. Miragoli, et al. [24] have shown the expression of connexins, between fibroblasts and, at contact sites, between fibroblasts and cardiomyocytes, by studying cocultured fibroblasts coated over rat-ventricular-myocyte strands; and Gaudesius, et al. [25] have reported that Cx43 and Cx45 are expressed among fibroblasts and between fibroblasts and myocytes when fibroblasts are inserted in cocultures of neonatal rat-heart cells in a monolayer.

Fibroblasts can play a major role in the propagation of electrical impulses in cardiac tissue. Some cell-culture [13,24–26] and in-silico [11,13,14] studies have reported the suppression of impulse propagation in cardiac tissue because of fibroblasts. For example, Miragoli, et al. [24] have studied electrical-impulse propagation in cultured strands of myocytes coated by fibroblasts and shown that the conduction velocity $CV$ decreases by an amount that depends on the density of fibroblasts. The work of Gaudesius, et al. [25] has demonstrated that conduction delay occurs because of the insertion of fibroblasts between myocytes in cultured myocyte strands; the delay depends on the number of inserted fibroblasts; and finally conduction block occurs when the length of the inserted fibroblasts exceeds 300 $\mu$m. Zlocchiver, et al. [13] have studied the propagation of electrical impulses in a monolayer of myocytes and fibroblasts of neonatal rats; in one set of experiments they have either increased or decreased the gap-junction coupling by overexpressing Cx43 or by using silencing RNAi; in another set of experiments they have varied the ratio of fibroblasts to myocytes. In the former case, they have observed that an increase in the gap-junctional conductance first leads to a decrease in $CV$ and then an increase; in the second set of experiments they have
<table>
<thead>
<tr>
<th>References</th>
<th>Type of studies</th>
<th>Type of fibroblast</th>
<th>Parameter ranges</th>
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<tbody>
<tr>
<td>Rook, et al. [5]</td>
<td>in culture</td>
<td>rat</td>
<td>( R_f \simeq 25 \Omega )</td>
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<td></td>
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<td>( E_f \simeq -20a - 40 , mV )</td>
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<tr>
<td>Kohi, et al. [3]</td>
<td>in vitro</td>
<td>rat</td>
<td>( R_f \simeq 1 \Omega )</td>
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<td></td>
<td>and</td>
<td></td>
<td>( E_f \simeq -15 \pm 10 , mV )</td>
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<td></td>
<td>in culture</td>
<td></td>
<td>( G_{gap} \simeq 3 - 6 , nS )</td>
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<tr>
<td>Kiseleva, et al. [46]</td>
<td>in vitro</td>
<td>rat</td>
<td>( R_f \simeq 0.51 \pm 0.01 \Omega ) (control case)</td>
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<td>( E_f \simeq -22 \pm 1.9 , mV ) (control case)</td>
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<td>( E_f \simeq -46 \pm 1.8 , mV ) (diseased case)</td>
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<tr>
<td>Kamkin, et al. [47]</td>
<td>in vitro</td>
<td>human</td>
<td>( R_f \simeq 4.1 \pm 0.1 \Omega )</td>
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<td>( E_f \simeq -15.9 \pm 2.1 , mV )</td>
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<tr>
<td>Kamkin, et al. [48]</td>
<td>in vitro</td>
<td>rat</td>
<td>( R_f \simeq 510 \pm 10 \Omega ) (control case)</td>
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<td>( E_f \simeq -22 \pm 2 , mV ) (control case)</td>
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<td>( E_f \simeq -41 \pm 3 , mV ) to ( -28 \pm 3 , mV ) (diseased case)</td>
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<tr>
<td>Chilton, et al. [49]</td>
<td>in culture</td>
<td>rat</td>
<td>( C_{f,0} \simeq 6.3 \pm 1.7 , pF )</td>
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<td>( R_f \simeq 10.7 \pm 2.3 \Omega )</td>
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<td></td>
<td>( E_f \simeq -65 \pm 5 , mV ) (for ( [K^+]_o = 10 , mM ))</td>
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<td>( \simeq -80 \pm 1.8 , mV ) (for ( [K^+]_o = 5.4 , mM ))</td>
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<tr>
<td>Shibukawa, et al. [50]</td>
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<td>rat</td>
<td>( C_{f,0} \simeq 4.5 \pm 0.4 , pF )</td>
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<td>( R_f \simeq 5.5 \pm 0.6 \Omega )</td>
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<td>( E_f \simeq -58 \pm 3.9 , mV )</td>
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<tr>
<td>Xie, et al. [9]</td>
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<td>passive</td>
<td>( C_{f,0} \simeq 25 , pF )</td>
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<td>( G_f \simeq 0.1 - 4 , nS )</td>
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<td>( E_f \simeq -50 - 0 , mV )</td>
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<td>( G_{gap} \simeq 0 - 20 , nS )</td>
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<td>( 0 \leq N_f \leq 4 )</td>
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<tr>
<td>Sachse, et al. [10]</td>
<td>computational</td>
<td>active</td>
<td>( C_{f,0} \simeq 4.5 , pF )</td>
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<td>( E_f \simeq -58 , mV )</td>
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<td>( G_{gap} \simeq 0.1 - 100 , nS )</td>
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<td>( 0 \leq N_f \leq 10 )</td>
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<tr>
<td>Jacquemet, et al. [11]</td>
<td>computational</td>
<td>active</td>
<td>( C_{f,0} \simeq 4.5 , pF )</td>
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<td>( E_f \simeq -58 , mV )</td>
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<td>( G_{gap} \simeq 0.09 - 4.05 , nS )</td>
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<td>( 0 \leq N_f \leq 10 )</td>
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<tr>
<td>MacCannell, et al. [12]</td>
<td>computational</td>
<td>active</td>
<td>( C_{f,0} \simeq 6 - 60 , pF )</td>
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<td>( E_f \simeq -49.6 , mV )</td>
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<td>( G_{gap} \simeq 1 - 3 , nS )</td>
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<td>( 0 \leq N_f \leq 4 )</td>
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Table 1. Cardiac fibroblast parameter values used in various experimental and computational studies.

found that \( CV \) decreases as the fibroblast density increases. McSpadden, et al. [26] have studied electrical-wave propagation in a monolayer of neonatal rat cardiac myocytes electrotonically loaded with a layer of cardiac fibroblasts; they have used an optical-mapping technique to find the dependence of such impulse propagation on the gap-junctional conductance \( G_{gap} \); and they have found that impulse propagation, in both the transverse and longitudinal directions, changes significantly when fibroblasts are loaded on the myocyte monolayers; e.g., as the fibroblast coverage area increases from the 0–15% coverage range to the 75–100% coverage range, the conduction velocity \( CV \), in loaded monolayers, decreases from \( \simeq 28 \pm 5 \, cm/s \) to \( \simeq 21 \pm 7 \, cm/s \) in the longitudinal direction, and from \( 13 \pm 3 \, cm/s \) to \( 9 \pm 3 \, cm/s \) in the transverse direction.

Xie, et al. [14] have followed Ref. [27] to model MF tissue in three different ways, namely, with (a) zero-sided, (b) single-sided, and (c) double-sided connections, by using the LRI [20] ventricular-cell model for myocytes with slight modifications of
the original parameters. In their zero-sided connection model, passive fibroblasts are inserted in a 2D layer of myocytes; but they are functionally uncoupled with myocytes at their contact sites, so the fibroblasts are equivalent to conduction inhomogeneities [16,17,23–30]. In the single-sided connection model, connected fibroblasts are loaded on the top of a 2D layer of myocytes; therefore, they are equivalent to an extra, local electrical load. In the double-sided-connection model, connected fibroblasts are inserted in a 2D myocardial layer, with myocytes and fibroblasts connected at contact sites; this provides an additional conduction pathway for electrical signals, so the fibroblasts are qualitatively similar to ionic inhomogeneities [16,17,31]. Their studies of fibroblasts randomly attached on the top of a 2D myocardial sheet (i.e., single-sided connections), show that, for low fibroblast membrane conductances $G_f \leq 1$ and with the fibroblast resting-membrane potential $E_f = -20$ mV, CV initially remains almost unchanged as the fibroblast-myocyte (FM) ratio increases; but then it decreases quickly as the FM ratio approaches 3. If, however, $G_f \geq 4$, CV increases initially and then decreases as the FM ratio approaches 1. However, in both cases, with low and high values of $G_f$, conduction failure occurs when CV decreases to $\sim 0.2$ m/s from its uncoupled value 0.56 m/s. Furthermore, when $E_f = -80$ mV (i.e., close to the myocyte resting-membrane potential), CV decreases linearly from 0.56 m/s to 0.49 m/s as the FM ratio increases from 0 to 3; this trend is almost independent of the value of $G_f$. They have also studied the effects of the random insertion of fibroblasts in a 2D sheet of myocytes sheet; the resulting myocyte-fibroblast pairs can have zero-sided or double-sided connections. When fibroblasts are inserted in series, CV decreases almost linearly as the FM ratio increases, for zero-sided connections, and conduction failure occurs if the FM ratio is above 3. Similar results are observed with double-sided connection when fibroblasts, with $E_f = -20$ mV and a low value of $G_f$ (1 nS) are coupled with myocytes. However, for larger values of $G_f$ (4 nS), CV decreases much faster as the FM ratio increases, and conduction failure occurs if the FM ratio is below 1. Furthermore, if $E_f = -80$ mV, CV is only slightly different from that with uncoupled fibroblasts and almost independent of $G_f$. If the fibroblasts lie parallel to myocytes in a 2D sheet, they have found that, with random laterally inserted fibroblasts coupled to all neighboring cells (double-sided connection), CV changes in both longitudinal and transverse directions, but to a different extent. In the longitudinal direction, CV is similar to that in models with random fibroblast attachment; however, in the transverse direction, CV decreases much more rapidly, as in to models with random fibroblast insertions. These authors have also studied the effects of $G_f$ on CV for randomly inserted fibroblasts (double-sided connection) in a 2D sheet of myocytes. For low $G_f$ (0.1 nS), with an FM ratio of 1, they have found that CV decreases first and then increases as $G_f$ increases ($G_f > 25$ nS). However, for high $G_f$ (2 nS), CV increases to a maximum (at $G_f \geq 5$ nS), then decreases to minimum (at $G_f \geq 25$ nS), and eventually increases linearly as $G_f$ increases.

Zlochiver, et al. [13] have studied impulse propagation, by inserting fibroblasts, in a 2D sheet of myocyte tissue in the dynamic Luo-Rudy (LRd) [32,33] model of a mammalian ventricular cell [34]. Their studies show that CV first increases and then decreases as $G_{gap}$ increases and then decreases as a function of fibroblast-myocyte area ratio, in agreement with their experimental observations.

The remaining part of this paper is organized as follows. In the Section on “Model and Methods” we describe the formulation of our myocyte-fibroblast model, for a single cell and for 2D tissue; we also describe the numerical schemes that we use to solve the model equations. In the Section on “Results” we present the results of our numerical calculations. In the Section on “Discussion and Conclusion” we discuss the significance of our results and compare them with results from other experimental and computational studies.

Model and Methods

In this Section, we build on earlier mathematical models for (a) cardiac tissue [15] and (b) the coupling, at the level of single cells, of cardiac myocytes and cardiac fibroblasts [9–12] to develop a mathematical model for a 2D sheet of cardiac myocytes coupled to a similar sheet of fibroblasts. We use the ionic model for human cardiac myocytes [15] due to ten Tusscher, Noble, Noble, and Panfilov (TNNP); we include connections between myocytes and fibroblasts via gap junctions; and we also allow for the possibility of studying zero-sided, one-sided, and two-sided couplings as illustrated in the schematic diagram of Fig. S1 in Material S1.

The cell membrane of a cardiac myocyte is modelled by the following ordinary differential equation (ODE) [35,36]

$$C_m \frac{\partial V_m}{\partial t} = -I_{ion,m} + I_{ext},$$

here $C_{m,\text{tot}}$ is the total cellular capacitance, $V_m$ is the transmembrane potential, i.e., the voltage difference between intra- and extra-cellular spaces, $I_{ion,m}$ is the sum of all ionic currents that cross the cell membrane, and $I_{ext}$ is the externally applied current. Similarly, the membrane potential of a passive fibroblast is given by the ODE

$$C_{f,\text{tot}} \frac{\partial V_f}{\partial t} = -I_{ion,f},$$

where $C_{f,\text{tot}}, V_f,$ and $I_{ion,f}$ are, respectively, the total cellular capacitance, the transmembrane potential, and the sum of all ionic currents for the fibroblast. The passive nature of the fibroblasts allows us to write

$$I_{ion,f} = G_f (V_f - E_f),$$

here $G_f$ and $E_f$ are, respectively, the conductance and the resting membrane potential for the fibroblast. If a single myocyte cell is coupled with $N_f$ fibroblasts via the gap junctional conductance $G_{gap}$, its transmembrane potential can be modelled by the following set of equations:

$$C_{m,\text{tot}} \frac{\partial V_m}{\partial t} = -I_{ion,m} + \sum_{n}^{} N_f I_{gap,n},$$

$$C_{f,\text{tot,n}} \frac{\partial V_{f,n}}{\partial t} = -I_{ion,f,n} - I_{gap,n},$$

where

$$I_{gap,n} = G_{gap} (V_{f,n} - V_m),$$

here $n$ labels the fibroblasts that are connected to the myocyte via $G_{gap}$; note that $1 \leq n \leq N_f$ and, for the identical fibroblasts we
consider here, \( C_{\text{m,tot}} = C_{\text{f,tot}} \), \( I_{\text{gap,n}} = I_{\text{gap}} \), and \( V_{f,n} = V_f \), for all \( n \). The physical units that we use for our model are as follows: time \( t \) is in ms, the transmembrane potentials \( V_m \) and \( V_f \) are in mV, the transmembrane currents \( I_{\text{ion,m}} \) and \( I_{\text{ion,f}} \) are in pA, therefore, current densities for the myocyte are in \( \mu A/\mu F \), the total cellular capacitances \( C_{\text{m,tot}} \) and \( C_{\text{f,tot}} \) are in \( \mu F \), and the fibroblast conductance \( G_f \) and the gap-junctional conductance \( G_{\text{gap}} \) are in \( nS \).

As suggested in Ref. [37], the dynamics of \( N_f \) identical fibroblasts coupled to a myocyte is equivalent to the dynamics of a single fibroblast coupled to a myocyte with coupling strength, \( G_{\text{mf}} = G_{\text{fm}} / N_f \), where \( G_{\text{mf}} \) is the coupling strength of a myocyte to \( N_f \) fibroblasts and \( G_{\text{fm}} = G_{\text{gap}} \) is the coupling strength of a fibroblast to a myocyte. Therefore, we have performed simulations by using only one fibroblast per myocyte in our 2D simulation domain. This is equivalent to a myocyte being coupled with \( N_f \) fibroblasts with coupling strength \( G_{\text{mf}} = G_{\text{fm}} / N_f \). Furthermore, in our 2D model, the maximum number of fibroblasts \( N_f_{\text{max}} \) allowed per site is roughly related with the ratio of \( C_{\text{m,tot}} \) and \( C_{\text{f,tot}} \) because they are related to the surface area of the cell; in experiments, \( N_f \) depends on the ratio of these surface areas and the volume fractions of myocytes and fibroblasts. These considerations are important because fibroblasts are considerably smaller than myocytes as we discuss in greater detail in the section on “Discussion and Conclusion”.

In our 2D computational studies, we use a simulation domain in which we have one layer of fibroblasts on top of a myocyte layer as illustrated in Fig. S1 in Material S1. Such a simulation domain is motivated by the experiments of Refs. [6–8,38]. We model this myocyte-fibroblast bilayer by using the following discrete equations [39,40]:

\[
C_{\text{m,tot}} V_m(i,j) = -I_{\text{ion,m}}(i,j) + G_{\text{gap}}(V_f(i,j) - V_m(i,j)) + \sum_{j=-1}^{1} G_{\text{nm}}(V_m(i+j) - V_m(i,j)) + \sum_{j=-1}^{1} G_{\text{fm}}(V_f(i) - V_m(i,j))
\]

\[
G_{\text{mf}}(V_f(i) - V_m(i,j)) + \sum_{j=-1}^{1} G_{\text{ff}}(V_f(i+j) - V_f(i,j))
\]

\[
G_{\text{ff}}(V_f(i+j) - V_f(i,j))
\]

here the dots above \( V_m \) and \( V_f \) denote time derivatives, \( G_{\text{nm}} \) and \( G_{\text{ff}} \) represent, respectively, intercellular couplings in the myocyte and fibroblast layers; and \( G_{\text{mf}} \) and \( G_{\text{fm}} \) account for cross couplings between myocyte and fibroblast layers; if one of the cross-coupling coefficients, say \( G_{\text{mf}} \), is nonzero, then the other, \( G_{\text{fm}} \), must also be nonzero to ensure current conservation; myocyte and fibroblast composites are coupled at a given site by \( G_{\text{gap}} \); in addition, we allow for intercellular couplings (see Fig. S1 in Material S1) that can be categorized naturally as follows: (A) zero-sided: \( G_{\text{nm}} > 0, G_{\text{mf}} = G_{\text{ff}} = G_{\text{fm}} = 0 \); (B) one-sided: \( G_{\text{nm}}, G_{\text{ff}} > 0, G_{\text{mf}} = G_{\text{fm}} = 0 \); and (C) two-sided: \( G_{\text{nm}}, G_{\text{ff}} > 0, G_{\text{mf}}, G_{\text{fm}} > 0 \); the index \((i,j)\) refers to the cell associated with the node under consideration; the conductances \( G_{\text{nm}}, G_{\text{ff}}, G_{\text{mf}}, G_{\text{fm}}, \) and \( G_{\text{gap}} \) are in \( nS \). Given that fibroblasts are much smaller than myocytes, the terms \( G_{\text{fm}}, G_{\text{mf}}, \) and \( G_{\text{ff}} \) are negligible relative to \( G_{\text{nm}} \) unless a cluster of \( N_f \) fibroblasts is formed, with \( N_f \) large enough for the size of the cluster to be comparable to that of a myocyte; such clusters can couple to myocytes and to each other and lead to a realization of the one- and two-sided models mentioned above (see Fig. S1 in Material S1); and for a schematic diagram of connections between a myocyte and a cluster of \( N_f = 6 \) fibroblasts see Fig. 7 in Ref. [37]; in such an effective model, we assume, at the level of the simplest approximation, that the fibroblasts in a cluster interact only to the extent that they form the cluster.

We use a 2D square domain consisting of \( 600 \times 600 \) grid points and lattice spacing \( \Delta x = \Delta y = 0.225 \) mm, so the side of each square domain is \( L = 135 \) mm; one of these layers contains myocytes and the other fibroblasts as shown in Fig. S1 in Material S1. These two layers are separated by a distance \( \Delta z = 0.225 \) mm. We use a forward-Euler method for the time evolution of the transmembrane potentials with a time step \( \Delta t = 0.02 \) ms. We use no-flux (Neumann) boundary conditions on the edges of the simulation domain. The initial condition we use is related to the one given in Ref. [17]; we describe it in detail in subsection 2 in the section on “Results”.

It is often useful to track the trajectory of the tip of a spiral wave to investigate the stability of a spiral, its transitions, and its rate of drifting in a 2D simulation domain. The tip of such a spiral wave is normally defined as the point where the excitation wave front and repolarization wave back meet; this point can be found as the point of intersection of an isopotential line, of constant membrane potential, \( V_m = V_{\text{tot}} \) (in general \( V_{\text{tot}} < 0 \) mV), and the line \( dV_m/dt = 0 \) [15,41,42]. Another classical technique, which tracks the spiral-wave tip in a two-variable model, obtains this tip by finding the point where the isocontours of the two state variables intersect [42–45]; this technique can also be used in a complex mathematical model for cardiac tissue provided the model has at least one slow and one fast variable. We have developed a tip-tracking algorithm that locates the tip position, e.g., the point at which the wave-front and wave-back meet each other, by monitoring \( I_{\text{Na}} \), the sodium current. This is the predominant current in the depolarization phase of the AP and is, therefore, responsible for depolarizing the cells that lie ahead of the wave front, in the 2D simulation domain; thus, it plays an important role in the spatiotemporal evolution of this wave front. Hence, we find the minimum strength of \( I_{\text{Na}} \) that can yield an AP; and we use this as a reference value to track the tip position. Given the sharpness of the depolarization, pseudocolor plots of \( I_{\text{Na}} \) show a fine line along a spiral-wave arm (see, e.g., Fig. 2A in Ref. [16]); this fine line stops at the spiral tip and provides, therefore, an accurate way of tracking the spatio-temporal evolution of this tip.

**Results**

In our previous studies [16,17], we have investigated the interaction of a spiral-wave with conduction and ionic inhomogeneities in the TNNP model for cardiac tissue. Here we elucidate spiral-wave dynamics in the presence of fibroblasts by using the mathematical model we have developed in the section on “Model and Methods”. In subsection 1.1 we present results for the morphology of the action potential (AP) in a myocyte-fibroblast (MF) composite; in particular, we examine the dependence of the AP on \( G_{\text{gap}}, G_{\text{ff}}, G_{\text{mf}}, G_{\text{fm}}, \) and \( N_f \). Subsection 2 contains our results for spiral-wave dynamics in a homogeneous MF bilayer, in which MF composites are coupled; we consider zero-, one-, and two-sided couplings. In subsection 2 we explore the dynamics of
spiral waves in a sheet of myocytes with an inhomogeneity that is an MF bilayer. The last subsection 3 examines the efficacy of the low-amplitude, mesh-based control scheme of Refs. [16,18,19] in the elimination of spiral waves in the homogeneous MF bilayer and the sheet of myocytes with an MF-bilayer inhomogeneity.

1 A Myocyte-Fibroblast (MF) Composite

Fibroblast cells act like an electrical load on myocytes. This load, which depends, principally, on the parameters \( G_{\text{gap}}, G_{f}, E_{f}, C_{f,\text{tot}}, \) and \( N_{f} \), and alters the electro-physiological properties of a myocyte that is coupled to a fibroblast. In particular, it modifies the morphology of the action potential (AP). Earlier computational studies [9–12], which we have summarized in the “Introduction” section, have investigated mathematical models for a single unit of a myocyte and fibroblasts, for both animal and human ventricular cells and with passive or active fibroblasts. Most of these computational studies focus on the modification of the AP by (a) the number \( N_{f} \) of fibroblasts per myocyte and (b) the gap-junctional conductance \( G_{\text{gap}} \). In the numerical studies that we present here we use a composite myocyte-fibroblast (MF) system with \( N_{f} \) passive fibroblasts per myocyte. We examine in detail the dependence of the AP of this composite on the parameters of the model, namely, the membrane capacitance \( C_{f,\text{tot}} \), the membrane conductance \( G_{f} \), the resting membrane potential \( E_{f} \), and the coupling strength \( G_{\text{gap}} \); the trends we uncover are in qualitative agreement with various experiments [3,5].

The ranges of parameters, which we use for our composite MF system, are consistent with those found in experimental studies [3,5,46–50] and those used in earlier computational studies [9–12] as we summarize in Table 1 and discuss in detail in Material S1. To investigate in detail the effect of fibroblasts on a myocyte, we use the following wide ranges of parameters (these encompass the ranges used in the experimental and computational studies mentioned in Table 1): \( C_{f,\text{tot}} = 6–60 \ pF, \ G_{f} = 0.1–4 \ nS, \ E_{f} = -39 \) to \( 0 \) mV, and \( G_{\text{gap}} = 0.3–8.0 \) nS for our MF composites. However, to observe some special properties, such as autorhythmicity of MF composites, we vary the fibroblast parameters and gap-junctional conductances.

Figures 1 (a)-(i) show plots of the myocyte transmembrane potential \( V_{m} \) (filled symbols with solid lines) and the fibroblast transmembrane potential \( V_{f} \) (unshaded symbols with dashed lines) versus time \( t \), when we consider an MF composite in which a myocyte is coupled to a passive fibroblast, with \( C_{f,\text{tot}} \approx 6 \ pF \). In Figs. 1 (a)-(i) we use squares ( ■ or □) for low coupling \( (G_{\text{gap}} = 0.1 \) nS), diamonds ( ◇ or ◆) for intermediate coupling

Figure 1. Plots of action potentials: The myocyte action potential \( V_{m} \) (full symbols and lines) and the fibroblast action potential \( V_{f} \) (unshaded symbols and dashed lines), with a passive fibroblast of capacitance \( C_{f,\text{tot}} = 6.3 \) pF coupled with a myocyte for (a) \( E_{f} = 0 \) mV and \( G_{f} = 0.1 \) nS, (b) \( E_{f} = -19 \) mV and \( G_{f} = 0.1 \) nS, (c) \( E_{f} = -39 \) mV and \( G_{f} = 0.1 \) nS, (d) \( E_{f} = 0 \) mV and \( G_{f} = 1 \) nS, (e) \( E_{f} = -19 \) mV and \( G_{f} = 1 \) nS, (f) \( E_{f} = -39 \) mV and \( G_{f} = 1 \) nS, (g) \( E_{f} = 0 \) mV and \( G_{f} = 4 \) nS, (h) \( E_{f} = -19 \) mV and \( G_{f} = 4 \) nS, and (i) \( E_{f} = -39 \) mV and \( G_{f} = 4 \) nS; red squares (full or unshaded) indicate \( G_{\text{gap}} = 0.3 \) nS; blue diamonds (full or unshaded) indicate \( G_{\text{gap}} = 1 \) nS; gray triangles (full or unshaded) indicate \( G_{\text{gap}} = 3 \) nS; black squares (full or unshaded) indicate an uncoupled myocyte.

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Figure 2. Plots of various morphological features of the myocyte action potential $V_m$ versus the gap-junctional conductance $G_{gap}$. (a) The action-potential duration APD versus $G_{gap}$, (b) the resting-membrane potential $V_{rest}$ versus $G_{gap}$, (c) the maximum upstroke velocity $dV/dt_{max}$ versus $G_{gap}$, (d) the maximum value of $V_m$ during the action potential, $V_{max}$ versus $G_{gap}$, (e) the value of $V_m$ at the position of the notch, i.e., $V_{notch}$ versus $G_{gap}$, (f) the maximum value of $V_m$ in the plateau region of the action potential, i.e., $V_{plateau}$ versus $G_{gap}$; these figures show plots for the fibroblast resting membrane potential $E_f = 0$ mV (full red triangles), $E_f = -9$ mV (full blue squares), $E_f = -19$ mV (full black circles), $E_f = -29$ mV (full black diamonds), and $E_f = -39$ mV (full red stars).

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$(G_{gap} = 1.0 \text{nS})$, triangles (△ or Δ) for high coupling $(G_{gap} = 8.0 \text{nS})$, and filled circles (●) for a myocyte that is not coupled to a fibroblast. Figures 1 (a), (d), (g), which are in the first column, depict $V_m$ and $V_f$ for low (0.1 nS), intermediate (1.0 nS), and high (4.0 nS) values of $G_f$ when $E_f = 0.0$ mV; their analogs for $E_f = 19.0$ mV and $-39.0$ mV are given, respectively, in Figs. 1 (b), (c), (b) second column) and Figs. 1 (c), (f), (b) (third column). These figures show the following: i) the fibroblast action potential (FAP) is similar to the myocyte action potential (mAP) when the gap-junctional conductance $G_{gap}$ is high and the fibroblast conductance $G_f$ is low (Figs. 1 (a), (b), (c) in the first row); ii) for low and intermediate values of $G_{gap}$ and with $G_f = 0.1$ nS, the FAP plateau decreases but the APD is prolonged with respect to that of the corresponding mAP; iii) the FAP loses its spike and notch and has a lower plateau and prolonged APD compared to the mAP when $G_f = 1.0$ or 4.0 nS. Figures similar to Figs. 1 (a)-(i), but with $C_{f,tot} = 25.2$ pF and $C_{f,tot} = 63$, are given, respectively, in Figs. S2 (a)-(i) and Figs. S3 (a)-(i) in Material S1; these show that $V_m$ does not depend very significantly on $C_{f,tot}$ but $V_f$ does.

In Figs. 2 (a), (b), (c), (d), (e), and (f) we show, respectively, plots of the action-potential duration APD, the resting-membrane potential $V_{rest}$, the maximum upstroke velocity $dV/dt_{max}$, the maximum value of $V_m$, during the action potential, namely, $V_{max}$, the value of $V_m$ at the position of the notch, i.e., $V_{notch}$, and the maximum value of $V_m$ in the plateau region of the action potential, i.e., $V_{plateau}$ versus the gap-junctional conductance $G_{gap}$; here the myocyte is coupled with a passive fibroblast with capacitance $C_{f,tot} = 6.3$ pF and conductance $G_f = 4.0$ nS. These figures show plots for the fibroblast resting membrane potential $E_f = 0$ mV (full red triangles), $E_f = -9$ mV (full blue squares), $E_f = -19$ mV (full black circles), $E_f = -29$ mV (full blue diamonds), and $E_f = -39$ mV (full red stars). For $-39 \text{mV} \leq E_f \leq -19 \text{ mV}$, the APD decreases monotonically as $G_{gap}$ increases, but for higher values of $E_f$, namely, $-9 \text{ mV}$ and $0 \text{ mV}$ their is a monotonic increase of the APD with $G_{gap}$. Both $dV/dt_{max}$ and $V_{max}$ decrease monotonically as $G_{gap}$ increases; the lower the value of $E_f$, the slower is this decrease. Similarly, $V_{notch}$ and $V_{plateau}$ decrease monotonically as $G_{gap}$ increases; but the higher the value of $E_f$, the slower is this decrease.

In Fig. 3 (a) we present plots versus time $t$ of the transmembrane potentials $V_m$ (full curves with filled symbols), for a myocyte, and $V_f$ (dashed curves with open symbols), for a passive fibroblast coupled with a myocyte cell; here $C_{f,tot} = 6.3$ pF, $G_f = 4.0$ nS, $G_{gap} = 8.0$ nS and $E_f = -39.0$ mV (blue squares) and $E_f = 0.0$ mV (red triangles); the full black curve with circle show, for comparison, a plot of $V_m$ for an uncoupled myocyte. Figure 3 (b) contains plots versus $t$ of the gap-junctional current $I_{gap}$ with parameters and symbols as in (b); and plots versus $t$ of $V_m - I_{gap}$, the myocyte sodium current $I_{Na}$, and the total activation $m^2$ (full lines with filled symbols) and total inactivation $h_f$ (dashed lines with open symbols) gates, for the first 2 ms after the application of a stimulus current $I_{ext} = -52 \text{ pA/pF}$ at 50 ms for 3 ms, are depicted in Figs. 3 (c), (d), (e), and (f), respectively, for the parameters and symbols used in Fig. 3 (a). These plots show that the myocyte membrane potential $V_m$ is reduced, i.e., the cell is depolarized, when a passive fibroblast is coupled with it; the larger the value of $E_f$, the more is the reduction in $V_m$. However, $V_m$ is elevated, compared to its value in the uncoupled-myocyte case, for both the values of $E_f$ we study, because, when $I_{ext}$ from $0 \leq t \leq 50 \text{ ms}$, the gap-junctional current $I_{gap}$ flows from the fibroblast to the myocyte as shown in Fig. 3 (b). The greater the elevation of $V_m$ the earlier is the activation of the $Na^+$ fast-activation gate $m$, in the presence of an external applied stimulus,
as can be seen by comparing Figs. 3 (c) and (f); this early activation shifts the minimum in \( I_{Na} \) towards the left as depicted in Fig. 3 (c).

Note that the product \( h_j \), the total inactivation gating variables, decreases as \( V_m \) increases (dashed lines in Fig. 3 (b)) in the range \( 50 \text{ ms} \leq t \leq 52 \text{ ms} \). Therefore, the amplitude of \( I_{Na} \) decreases with increasing \( E_f \), as shown in Fig. 3 (c), and leads to a reduction in the maximum rate of AP depolarization (see the plots of \( dV/dt_{\text{max}} \) in Fig. 2 (c)); this shift in the minimum of \( I_{Na} \) is also associated with the leftward shift of \( V_m \) in Fig. 3 (c). A comparison of Figs. 3 (c) and (f) shows, furthermore, that, for a given value of \( E_f \), the minimum of \( I_{Na} \) occurs at the value of \( t \) where the plots of \( h_j \) cross.

The current \( I_{gap} = G_{gap}(V_m - V_f) \) flows from the myocyte to the fibroblast or vice versa as shown in Figs. 3 (b) and (d). Before the application of a stimulus current (0 ms \( \leq t \leq 50 \text{ ms} \) in Fig. 3 (b)), \( I_{gap} < 0 \), i.e., it flows from the fibroblast to the myocyte; and the current-sink capability of the fibroblast increases with \( E_f \), because (see Fig. 3 (a)) \( (V_m - V_f) \) increases with \( E_f \). However, when we have a current stimulus \( I_{ext} > 0 \), i.e., in the time interval \( 50 \text{ ms} < t \leq 53 \text{ ms} \), the trend noted above is reversed: the lower the value of \( E_f \) the higher is the ability of the myocyte to act as a current sink as shown in Fig. 3 (d).

Several studies [31–54] have shown that the contribution of individual ionic currents to the AP morphology can be examined by a partial or complete blocking of the corresponding ion channel. Therefore, we examine, for an isolated myocyte, how the AP morphology changes as we modify the major ionic currents. As in Refs. [31–54], we find that (a) \( V_{\text{max}} \) and \( dV/dt_{\text{max}} \) depend principally on \( I_{Na} \); (b) \( V_{\text{match}} \) depends mainly on \( I_{Na} \); (c) the maximum of the plateau region \( V_{\text{plateau}} \) is maintained by a balance between \( I_{Ca,L} \) and \( I_{Kr} \); (d) the final phase of repolarization, which determines the APD, depends primarily on \( I_{Kr} \) and \( I_{Ks} \); (e) the diastolic or resting phase, which decides the value of \( V_{\text{rest}} \), is maintained predominantly by \( I_{K1} \), and (f) all gating variables, which determine the opening and closing of ion channels, depend on \( V_m \); therefore, the contribution of the ionic currents to the morphology of the AP is modified as \( V_m \) changes.

Given these results for an isolated myocyte, we can understand qualitatively the effects on the AP morphology of a myocyte when it is coupled with a fibroblast. The coupling of a fibroblast to a myocyte modifies \( V_m \) because of the electronic interaction, via \( G_{gap} \). Therefore, the AP morphology changes as we have described above and shown in Figs. 2 (a)–(f); to explain the results in this figure, we have to examine the behaviors of all the ionic currents when the myocyte is coupled to a fibroblast. For the ensuing discussion we consider a representative value of \( G_{gap} \), namely, 8.0 nS, and study the variation of the ionic currents as we change \( E_f \) for an MF composite. In particular, we examine the time-dependence of the myocyte ionic currents \( I_{Na}, I_{Ca,L}, I_{Na}, I_{Kr}, I_{Ks}, I_{Na}; I_{Na}, I_{Ca,L}, I_{Na}, I_{Ca,L}, I_{Na}, I_{Ca,L} \), which are plotted in Fig. 4 for a fibroblast coupled with a myocyte, with \( C_{f,tot} = 6.3 \text{ pF} \), \( G_{f} = 4.0 \text{ nS} \), \( G_{gap} = 8.0 \text{ nS} \) and \( E_f = -39.0 \text{ mV} \) (blue squares) and \( E_f = 0.0 \text{ mV} \) (red triangles); the full black curves with circles show the transmembrane potentials \( t \) for an uncoupled myocyte. (a) Plots versus time \( t \) of the transmembrane potentials \( V_m \) (full curves with filled symbols), for a myocyte, and \( V_f \) (dashed curves with open symbols), for a passive fibroblast coupled with a myocyte cell; here \( C_{f,tot} = 6.3 \text{ pF} \), \( G_f = 4.0 \text{ nS} \), \( G_{gap} = 8.0 \text{ nS} \) and \( E_f = -39.0 \text{ mV} \) (blue squares) and \( E_f = 0.0 \text{ mV} \) (red triangles); the full black curve with circles shows \( V_m \) for an uncoupled myocyte. (b) Plots versus \( t \) of the gap-junctional current \( I_{gap} \) with parameters and symbols as in (a). Plots versus \( t \), for the first 2 ms after the application of a stimulus of \( ~-52 \text{ pA/pF} \), of (c) \( V_m \), (d) \( -I_{gap} \). (e) the myocyte sodium current \( I_{Na} \), and (f) the total activation \( n^4 \) (full lines with filled symbols) and total inactivation \( h_j \) (dashed lines with open symbols); gates; the parameters and symbols are here as in (a). doi:10.1371/journal.pone.0072950.g003

Figure 3. Plots of transmembrane potentials and currents for an MF composite: (a) Plots versus time \( t \) of the transmembrane potentials \( V_m \) (full curves with filled symbols), for a myocyte, and \( V_f \) (dashed curves with open symbols), for a passive fibroblast coupled with a myocyte cell; here \( C_{f,tot} = 6.3 \text{ pF} \), \( G_f = 4.0 \text{ nS} \), \( G_{gap} = 8.0 \text{ nS} \) and \( E_f = -39.0 \text{ mV} \) (blue squares) and \( E_f = 0.0 \text{ mV} \) (red triangles); the full black curve with circles shows \( V_m \) for an uncoupled myocyte. (b) Plots versus \( t \) of the gap-junctional current \( I_{gap} \) with parameters and symbols as in (a). Plots versus \( t \), for the first 2 ms after the application of a stimulus current (\( = -39 \text{ mV} \)), for a myocyte, and \( V_m \), (d) \( -I_{gap} \), (e) the myocyte sodium current \( I_{Na} \), and (f) the total activation \( n^4 \) (full lines with filled symbols) and total inactivation \( h_j \) (dashed lines with open symbols); gates; the parameters and symbols are here as in (a). doi:10.1371/journal.pone.0072950.g003
Figure 4. Plots of ionic current, $I_{m}$, of the myocyte versus time $t$ of an MF composite with $N_f=1$; the fibroblast parameters are $C_{f, tot}=6.3$ pF and $G_f=4.0$ nS, and it coupled with a myocyte with $G_{gap}=8.0$ nS; the full black curve with circles shows $I_{m}$ for an uncoupled myocyte; the blue filled squares and the red triangles are, respectively, for $E_f=-39$ mV and $E_f=0$ mV. (a) The fast inward $Na^+$ current, $I_{Na}$; (b) the L-type slow inward $Ca^{2+}$ current, $I_{CaL}$; (c) the transient outward current, $I_{NaCa}$; (d) the plateau $Ca^{2+}$ current, $I_{plateau}$; (e) the plateau $K^+$ current, $I_{plateauK}$; (f) the background $Na^+$ current, $I_{NaB}$; (g) the background $Ca^{2+}$ current, $I_{CaB}$.

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decrease as $E_f$ increases (as shown in Figs. 2(c) and (d)). Similarly, we look at $I_{Na}$ to understand the dependence of $V_{notch}$ on $E_f$; and we examine $I_{CaL}$ and $I_{Kt}$ for the $E_f$-dependence of $V_{plateau}$. Figure 4(c) shows that $I_{Na}$ decreases when $E_f$ increases, therefore, $V_{notch}$ increases as a function of $E_f$ (as shown in Fig. 2(c)). Figures 4(b) and (d) show $I_{CaL}$ and $I_{Kt}$, respectively; the former decreases and the latter increases as $E_f$ increases; however, the effect of $I_{Kt}$ dominates that of $I_{CaL}$, so $V_{plateau}$ increases as $E_f$ increases as shown in Fig. 2(f).

It has been noted in Refs. [37,55,56], that a myocyte cell can display autorhythmicity when it is coupled with fibroblasts; in particular, Ref. [55] shows that the cycle length of autorhythmic activation depends on $E_f$ and $G_{gap}$. We find that $G_f$ and $C_{f, tot}$ play a less important role than $N_f$, $E_f$, and $G_{gap}$ in determining whether such autorhythmicity is obtained. We show that the myocyte can behave like (a) an excitable, (b) autorhythmic, or (c) oscillatory cell depending on the value of $G_{gap}$ as shown and discussed in detail in Fig. S4 in Material S1.

The number of fibroblasts $N_f$ that are coupled to a myocyte in our MF composite affect significantly the response of the MF composite to external electrical stimuli as has been shown in detail in Refs. [9,57]. In Fig. 5, we show illustrative plots versus $t$ of $V_m$, $V_f$, and $-I_{gap}$ for our MF composite with $N_f=0$, i.e., no fibroblasts (black circles), $N_f=1$ (red squares), $N_f=2$ (blue diamonds), and $N_f=4$ (gray triangles) for the parameter values $C_{f, tot}=6.3$ pF, $G_f=4$ nS, $E_f=-39$ mV. For a low value of $G_{gap}$, namely, 0.3 nS (plots in the left column), we see that the resting potential of the coupled myocyte is elevated slightly relative to the case $N_f=0$; and the APD decreases from 280 ms, for $N_f=0$, to 260 ms when $N_f=4$ (Fig.5(a)); the dependence of $V_f$ and $I_{gap}$ on $N_f$ is illustrated in Figs.5(d) and (g). This dependence of $V_m$, $V_f$, and $I_{gap}$ increases as we can see from the plots in the middle column, Figs.5(b), (c), and (h), for an intermediate value of $G_{gap}$, namely, 1 nS, and from the plots in the right column, Figs.5(c), (f), and (i), for an higher value of $G_{gap}$, namely, 8 nS. In the former case ($G_{gap}=1$ nS) $V_{rest}$ rises from $-84.6$ mV to $-83.2$ mV and the APD decreases from 280 ms to 240 ms as we go from $N_f=0$ to $N_f=4$ (Fig.5(b)); in the latter case ($G_{gap}=8$ nS) $V_{rest}$ rises from $-84.6$ mV to $-74.4$ mV and the APD decreases from 280 ms to 150 ms as we go from $N_f=0$ to $N_f=4$ (Fig.5(c)).

In Table 2, we show the change of the APD, $V_{max}$, and $V_{rest}$ for an MF composite with respect to their uncoupled values when $N_f$ identical fibroblasts are coupled with a myocyte with high value of both $G_f$ (4 nS) and $G_{gap}$ (8 nS). We measure $\Delta APD_{70\%}=APD_{70\%}-APD_{70\%}$, where $APD_{90\%}$ and $APD_{70\%}$ are, respectively, APD of an MF composite and isolated myocyte.
at 70% repolarization. Similarly, ΔAPD80 and ΔAPD90 are, respectively, the change in APD of MF composite with respect to the myocyte APD at 80% and 90% repolarization of AP. We do not present here the results of composites with Nf fibroblasts, which show AP automatically in the absence of external stimulus. The change of 

\[ V_{\text{max}} \] 

, Δ\[ V_{\text{max}} \] , is measured by subtracting \[ V^{\text{m}}_{\text{max}} \] of an MF composite form an isolated myocyte \[ V^{\text{m}}_{\text{max}} \]. Similarly, the change of \[ V_{\text{rest}} \] , Δ\[ V_{\text{rest}} \] , is measured by subtracting \[ V^{\text{m}}_{\text{rest}} \] of an MF composite form an isolated myocyte \[ V^{\text{m}}_{\text{rest}} \]. The analog of Table 2 for low values of \[ G_f \] (0.1 nS) and \[ G_{\text{gap}} \] (0.3 nS) is given in Table S1 in the Material S1.

2 Wave Dynamics in a 2D Simulation Domain with MF Composites

We move now to a systematic study of the propagation of electrical waves of activation in a 2D simulation domain with MF composites that are coupled via the types of intercellular and gap-junctional couplings described in the section on “Model and Methods”. We begin with an examination of plane-wave propagation through such a simulation domain and study the dependence of the conduction velocity \[ CV \] on the gap-junctional coupling \[ G_{\text{gap}} \] for zero-, one-, and two-sided couplings. We then study spiral-wave propagation in this domain. Finally, we investigate spiral-wave propagation through an inhomogeneous, square simulation domain in which most of the domain consists of myocytes, but a small region comprises a square MF composite.

In most of our numerical simulations of 2D MF composite domains, we choose the following representative values: the total cellular capacitance \[ C_f \text{ tot} \approx 6.3 \text{ pF}; \] the fibroblast conductance \[ G_f \approx 4.0 \text{ nS}; \] the resting membrane potential of fibroblast \[ E_f \approx -39 \text{ mV}; \] and \[ D_{\text{mm}} \approx 0.00154 \text{ cm}^2/\text{ms} \ [15], \] which yields, in the absence of fibroblasts, the maximum value for the plane-wave conduction velocity \[ CV \], namely, \(-68.3 \text{ cm/s} \ [17]; \) here \[ D_{\text{mm}} \] is the diffusion constant of the myocyte layer, and it is given by the relation, \[ D_{\text{mm}} = \frac{G_{\text{mm}} S_m}{C_{m\text{ tot}}}, \] where \[ S_m \] is the surface area of the myocyte. For the gap-junctional conductance we explore values in the experimental range (see section on “Introduction”), 0.0 nS ≤ \[ G_{\text{gap}} \] ≤ 8.0 nS; the remaining intercellular conductances lie in the following ranges: 0 ≤ \[ G_f \] ≤ \[ G_{\text{mm}}/200 \] and 0 ≤ \[ G_{\text{mf}} \] ≤ \[ G_{\text{mm}}/200 \]. In some of our studies, we vary \[ G_f \] and \[ E_f \]; e.g., when we study spiral wave dynamics in the autorhythmie regime, we use \[ G_f = 8.0 \text{ nS} \] and \[ E_f = 0.0 \text{ mV} \].

Spiral waves in homogeneous domains. As we have noted in the section on “Introduction”, both experimental and

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**Figure 5.** Plots versus \( t \) of \( V_m \), \( V_f \), and \( -I_{\text{gap}} \) for \( N_f = 0 \), i.e., no fibroblasts (black circles), \( N_f = 1 \) (red squares), \( N_f = 2 \) (blue diamonds), and \( N_f = 4 \) (gray triangles) for the illustrative parameter values \( C_f\text{ tot} = 6.3 \text{ pF}; \) \( G_f = 4 \text{ nS}; \) \( E_f = -39 \text{ mV}; \) and a low value of \( G_{\text{gap}} \), namely, 0.3 nS (plots in the left panel (a), (d), and (g)), an intermediate value of \( G_{\text{gap}} \), namely, 1.0 nS (plots in the middle panel (b), (e), and (h)), and a high value of \( G_{\text{gap}} \), namely, 8.0 nS (plots in the right panel (c), (f), and (i)).

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computational studies [13,14,26] have shown that CV behaves nonmonotonically as a function of the number of fibroblasts $N_f$ in an MF composite. However, to the best of our knowledge, no simulation has examined in detail the dependence of CV on $G_{gap}$; therefore, we examine this dependence for the zero-, single-, and double-sided couplings described in the section “Model and Methods”.

We measure CV for a plane wave by stimulating the left boundary of the simulation domain with a current pulse of amplitude 150 pA/pF for 3 ms. This leads to the formation of a boundary of the simulation domain with a current pulse of $G_{gap}$ for (g) zero-sided coupling, (h) single-sided coupling with $G_{mn}/G_{ff} = 1$, (i) double-sided coupling with $G_{mn}/G_{ff} = 1$, $G_{mn}/G_{ms} = 100$, and $G_{gap} = 0.5$ nS, (c) double-sided coupling with $G_{mn}/G_{ff} = 1$, $G_{mn}/G_{ms} = 100$, and $G_{gap} = 8.0$ nS, and (f) double-sided coupling with $G_{mn}/G_{ff} = 1$, $G_{mn}/G_{ms} = 200$, and $G_{gap} = 0.5$ nS. Video S1 illustrates the spatiotemporal evolution of these plane waves. Figures 6 (g)–(h) show plots of CV versus $G_{gap}$ for (g) zero-sided coupling, (h) single-sided coupling with $G_{mn}/G_{ff} = 1$ (blue circles), $G_{mn}/G_{ff} = 4$ (black squares), and $G_{mn}/G_{ff} = 100$ (red triangles), and (i) double-sided coupling with $G_{mn}/G_{ff} = 1$ and $G_{mn}/G_{ms} = 200$. For double-sided coupling, conduction failure can occur for low and intermediate values of $G_{gap}$, e.g., 0.5 nS and 2.0 nS, as shown in Fig. 6 (d); however, no conduction occurs in this range if $G_{mn}/G_{ms} = 200$. Such conduction failure is responsible for anchoring a spiral wave, at a localized MF composite, as we discuss in the next section where we describe our studies with inhomogeneities. We do not observe any conduction failure in the cases of zero- and one-sided coupling.

Two methods are often used to initiate spiral waves in simulations [13,16,58,59] and in experiments [58,60], namely, (1) the S1–S2 cross-field protocol and (2) the S1–S2 parallel-field protocol. In the cross-field method, a super-threshold stimulus $S_2$ is applied at the boundary that is perpendicular to the boundary along which the S1 stimulus is given; in the parallel-field method, S2 is applied parallel to the refractory tail of the S1 stimulus, but not over the entire length of the domain. We use the parallel-field protocol to initiate a spiral wave in our homogeneous, square simulation domain with myocytes as follows. Initially we set the diffusion constant $D_{mm} = 0.000385$ cm$^2$/ms; this is a quarter of its original value, which is 0.00154 cm$^2$/ms. We then inject a plane wave into the domain via an S1 stimulus of strength 150 pA/pF.

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Table 2. The values of $C_{f, tot}$, $G_f$, $G_{gap}$, $E_f$, and $N_f$ for a single MF composite and the changes in the AP morphology, relative to that of an uncoupled myocyte.

We concentrate on the APD, $V_{exc}$, and $V_{rest}$ and list the changes, indicated by Δ, in these parameters. $\Delta APD_{ms}$, $\Delta APD_{ms}$, and $\Delta APD_{ms}$ denote, respectively, the changes in the APD at 70%, 80%, and 90% repolarization. Note that here we have high values (see text) for both $G_f$ (4 nS) and $G_{gap}$ (8 nS).

We show plane waves in Figs. 6 (a)–(f) via pseudocolor plots of $V_m$ at $t = 160$ ms in a 2D square simulation domain of side $L = 135$ mm for (a) the control case with only myocytes, (b) zero-sided coupling, (c) single-sided coupling with $G_{mn}/G_{ff} = 1$, (d) double-sided coupling with $G_{mn}/G_{ff} = 1$, $G_{mn}/G_{ms} = 100$, and $G_{gap} = 0.5$ nS, (e) double-sided coupling with $G_{mn}/G_{ff} = 1$, $G_{mn}/G_{ms} = 100$, and $G_{gap} = 8.0$ nS, and (f) double-sided coupling with $G_{mn}/G_{ff} = 1$, $G_{mn}/G_{ms} = 200$, and $G_{gap} = 0.5$ nS. Video S1 illustrates the spatiotemporal evolution of these plane waves. Figures 6 (g)–(h) show plots of CV versus $G_{gap}$ for (g) zero-sided coupling, (h) single-sided coupling with $G_{mn}/G_{ff} = 1$ (blue circles), $G_{mn}/G_{ff} = 4$ (black squares), and $G_{mn}/G_{ff} = 100$ (red triangles), and (i) double-sided coupling with $G_{mn}/G_{ff} = 1$ and $G_{mn}/G_{ms} = 200$. For double-sided coupling, conduction failure can occur for low and intermediate values of $G_{gap}$, e.g., 0.5 nS and 2.0 nS, as shown in Fig. 6 (d); however, no conduction occurs in this range if $G_{mn}/G_{ms} = 200$. Such conduction failure is responsible for anchoring a spiral wave, at a localized MF composite, as we discuss in the next section where we describe our studies with inhomogeneities. We do not observe any conduction failure in the cases of zero- and one-sided coupling.
for 3 ms at the left boundary. After 560 ms we apply an S2 stimulus of strength 450 pA/pF for 3 ms just behind the refractory tail of the plane wave initiated by the S1 stimulus; in our simulation domain the S2 stimulus is applied over the region \( x = 360 \) and \( 1 \leq y \leq 550 \). We reset \( D \) to its original value after 882 ms. This procedure yields a fully developed spiral wave at \( t = 976 \text{ms} \) (see Fig. S5 in Material S1 for a pseudocolor plot of the transmembrane potential for this spiral wave); we use the values of \( V_m \), the gating variables, the intracellular ion concentrations for this spiral wave as the initial condition for our subsequent studies. By decreasing \( D \) for this small interval of time we are able to reduce \( CV \) (because \( CV \propto \sqrt{D} \)) and, thereby, the wave length \( \lambda \); if we do not reduce \( D \) for this period, it is difficult to trap the hook of the proto spiral simulation domain, whose side \( L = 13.5 \text{cm} \).

We use the fully developed spiral wave of Fig. S5 in Material S1 in (c) as an initial condition for the myocytes in our 2D simulation domain with MF composites; for the fibroblasts \( V_f \) is set equal to \( E_f \) at this initial time.

We begin by studying the dependence on \( G_{gap} \) of spiral-wave dynamics in our 2D MF-composite simulation domain with zero-sided coupling. In Fig. 7 we show pseudocolour plots of \( V_m \) in a square simulation domain of side \( L = 135 \text{mm} \), at time \( t = 2 \text{s} \) with \( G_f = 4.0 \text{nS}, E_f = -39.0 \text{mV} \), and (a) \( G_{gap} = 0.0 \text{nS} \) (control case, i.e., only myocytes), (b) \( G_{gap} = 0.5 \text{nS} \) (low coupling), (c) \( G_{gap} = 2.0 \text{nS} \) (intermediate coupling), and (d) \( G_{gap} = 8.0 \text{nS} \) (high coupling); the white solid lines show the trajectory of the spiral tip for \( 2 \text{s} \leq t \leq 3 \text{s} \). Video S2 illustrates the spatiotemporal evolution of these spiral waves. The plot in Fig. 7 (c) of the rotation period \( \tau_{rot} \) of the spiral wave versus \( G_{gap} \) shows that \( \tau_{rot} \) decreases as \( G_{gap} \) increases. In Fig. 7 (f) we show the power spectra \( E(\omega) \) of the time series of \( V_m(x,y,t) \) recorded from the representative point \( x = 112.5 \text{mm}, y = 112.5 \text{mm} \), which is indicated by an asterisk in Figs. 7 (a)-(d), for \( G_{gap} = 0.0 \text{nS} \) (black circles), (b) \( G_{gap} = 0.5 \text{nS} \) (gray squares), (c) \( G_{gap} = 2.0 \text{nS} \) (blue diamonds), and (d) \( G_{gap} = 8.0 \text{nS} \) (red triangles); for these power spectra we use time series with 400000 points; the discrete lines in these power spectra show that, over these time scales, we have periodic temporal evolution that is a characteristic signature of a single, rotating spiral wave. The position of the fundamental peak in these power spectra moves to high frequencies as we increase \( G_{gap} \) in a manner that is consistent with the decrease of \( \tau_{rot} \) in (c).

The rotation period \( \tau_{rot} \), for zero-sided coupling, decreases as \( G_{gap} \) increases. We can understand this qualitatively by looking at the APD of an MF composite and examining the propagation properties of plane waves that we have discussed above. Recall that, as \( G_{gap} \) increases, the myocyte APD for a myocyte in an MF composite decreases for low values of \( E_f \) (e.g., \( E_f = -39 \text{mV} \), as shown by red triangles in Fig. 2 (a)). The APD is roughly equal to the refractory time of a plane wave in a 1D or 2D domain [61], therefore, the refractory period of a plane wave in 2D decreases as
$G_{\text{gap}}$ increases; and we have checked explicitly that it decreases as $G_{\text{gap}}$ increases by applying an additional stimulus at the wave back of the propagating plane wave. The rotation period $\tau_{\text{rot}}$ of a spiral wave is related to the refractory period of a plane wave, although the curvature plays an additional role in the spiral-wave case [62,63]. Therefore, spiral waves rotate faster as $G_{\text{gap}}$ increases.

The white solid lines in Figs. 7(a)–(d) show the trajectories of the spiral tips for $2 \leq t \leq 3$ s. If we monitor these trajectories for longer durations of time, we obtain the tip trajectories shown in Figs. 8 (a), (b), and (c), respectively, for the time intervals $2 \leq t \leq 3$ s, $4 \leq t \leq 5$ s, and $6 \leq t \leq 7$ s for the case of zero-sided coupling, for the control case with $G_{\text{gap}} = 0.0$ ns (black circles), $G_{\text{gap}} = 0.5$ ns (gray squares), $G_{\text{gap}} = 2.0$ ns (blue diamonds), and $G_{\text{gap}} = 8.0$ ns (red triangles), and all other parameter values as in Fig. 7. Note that in Figs. 8 (a) and (b), i.e., for $t \leq 5$ s, these trajectories are very nearly circular for all the values of $G_{\text{gap}}$ we consider. However, for $t > 5$ s, the tip trajectories can form Z-type curves as shown in Fig. 8 (c) for the control case with $G_{\text{gap}} = 0.0$ ns (black circles), $G_{\text{gap}} = 0.5$ ns (gray squares), and $G_{\text{gap}} = 2.0$ ns (blue diamonds); the trajectory for $G_{\text{gap}} = 8.0$ ns (red triangles) continues to be circular. Videos S2, S3, and S4 illustrate the spatiotemporal evolution of these spiral-tip trajectories. The stability of the spiral core increases as we increase $G_{\text{gap}}$ because the interaction of the wave back of the preceding arm of the spiral wave and the wave front of the following arm decreases with increasing $G_{\text{gap}}$.

We also study spiral-wave dynamics in both autorhythmic and oscillatory regimes as shown and discussed in detail in Fig. S6 in Material S1.

In Fig. 9 we show spiral waves via pseudocolor plots of $V_m$ at time $t = 2$ s; here, at each site, we have an MF composite with a myocyte M coupled with one fibroblast F for which $G_{\text{gap}} = 8$ ns, $C_{\text{f.tot}} = 6.3$ pF, $G_f = 4$ nS, and $E_f = -39$ mV. Figure 9(a) shows the control case in which there are only myocytes and no

![Figure 7. Pseudocolour plots of $V_m$ with zero-sided couplings: $V_m$ in a square simulation domain of side $L = 135$ mm, at time $t = 2$ s with $G_f = 4.0$ nS, $E_f = -39.0$ mV, with $D_{\text{mm}} = 0.00154$ cm$^2$/s, and (a) $G_{\text{gap}} = 0.0$ nS (control case, i.e., only myocytes), (b) $G_{\text{gap}} = 0.5$ nS (low coupling), (c) $G_{\text{gap}} = 2.0$ nS (intermediate coupling), and (d) $G_{\text{gap}} = 8.0$ nS (high coupling); the white solid lines show the trajectory of the spiral tip for $2 \leq t \leq 3$ s. Video S2 illustrates the spatiotemporal evolution of these spiral waves. (e) Plot of the rotation period $\tau_{\text{rot}}$ of the spiral wave versus $G_{\text{gap}}$. (f) Plots of the power spectra $E(v)$ of the time series of $V_m(x,y,t)$ recorded from the representative point ($x = 112.5$ mm, $y = 112.5$ mm), which is indicated by an asterisk in (a)–(d), for $G_{\text{gap}} = 0.0$ nS (black circles), $G_{\text{gap}} = 0.5$ nS (gray squares), $G_{\text{gap}} = 2.0$ nS (blue diamonds), and $G_{\text{gap}} = 8.0$ nS (red triangles); for these power spectra we use time series with 400000 points. doi:10.1371/journal.pone.0072950.g007]

![Figure 8. Plots of spiral-wave-tip trajectories with zero-sided couplings: Spiral-wave tip trajectories for the time intervals (a) $2 \leq t \leq 3$ s, (b) $4 \leq t \leq 5$ s, and (c) $6 \leq t \leq 7$ s, with zero-sided coupling, and for the control case with $G_{\text{gap}} = 0.0$ ns (black circles), $G_{\text{gap}} = 0.5$ ns (gray squares), $G_{\text{gap}} = 2.0$ ns (blue diamonds), and $G_{\text{gap}} = 8.0$ ns (red triangles), and all other parameter values as in Fig. 7. Videos S2, S3, and S4 illustrate the spatiotemporal evolution of these spiral-tip trajectories. doi:10.1371/journal.pone.0072950.g008]
Figure 9. Spiral waves with zero-, one-, and two-sided couplings: Pseudocolor plots of $V_m$ at time $t=2$ s in a simulation domain with $L=13.5$ cm, an MF composite at every site, with a myocyte $M$ coupled via $G_{mm}=8$ nS with one fibroblast $F$ ($C_{tot}=0.3$ pF, $G_f=4$ nS, and $E_f=-39$ mV) for (a) control case with only myocytes and no fibroblasts, (b) zero-sided coupling, (c) single-sided coupling with $G_{mm}/G_{ff}=1$, and (d) double-sided coupling with $G_{mm}/G_{ff}=1$ and $G_{mm}/G_{ff}=200$; the white solid lines in these plots show the trajectories of the spiral tip for $2s<t<3s$. Video S5 illustrates the spatiotemporal evolution of these spiral waves. (e) Time series data for $V_m(x,y,t)$ are recorded at the point $(x=112.5$mm),$y=112.5$ mm), shown by an asterisk, for $6s<t<8s$. (f) Plot of the inter-beat interval (ibi) versus the beat number $n$, and (g) the power spectrum $E(n)$ of $V_m$ versus the frequency $n$ for the control case (black circles), zero-sided coupling (gray squares), single-sided coupling (blue diamonds), and double-sided coupling (red triangles); these plots of ibi and $E(n)$ are obtained from a time series of $V_m$ with 400000 data points separated by 0.02 ms. doi:10.1371/journal.pone.0072950.g009

fibroblasts; Fig. 9(b) shows the spiral wave for the case of zero-sided coupling; Fig. 9(c) gives the wave for single-sided coupling with $G_{mm}/G_{ff}=1$; and Fig. 9(d) portrays this wave when we have double-sided coupling with $G_{mm}/G_{ff}=1$ and $G_{mm}/G_{ff}=200$; the white solid lines in these plots show the trajectories of the spiral tip for $2s<t<3s$. Video S5 illustrates the spatiotemporal evolution of these spiral waves. In Fig. 9(d), we show the time series of $V_m(x,y,t)$, in the interval $68<t<88$, obtained from a representative point, shown by asterisks in Figs. 9(a–d), namely, $(x=112.5$mm),$y=112.5$ mm), for the control case (black circles), zero-sided coupling (gray squares), single-sided coupling (blue diamonds), and double-sided coupling (red triangles); Figs 9(e) and (f) contain, respectively, plots of the inter-beat interval (ibi) versus the beat number $n$ and the power spectrum $E(n)$ of $V_m$ versus the frequency $n$ for the control case (black circles), zero-sided coupling (gray squares), single-sided coupling (blue diamonds), and double-sided coupling (red triangles); these plots of ibi and $E(n)$ are obtained from a time series with $4 \times 10^5$ data points separated by 0.02 ms and recorded from the representative point $(x=112.5$mm),$y=112.5$ mm) that is indicated by a * in these pseudocolor plots. With double-sided couplings, we have seen the formation of two spirals (see Fig. 9(d)), given the spiral-initialization procedure that we use. From the animations in Video S5, the time series of $V_m(x,y,t)$, the ibi, and the power spectra in Fig. 9, we conclude that the spiral rotates faster for single-sided coupling than for zero-sided coupling; the spiral rotation rate in the double-sided case lies between these rates for zero- and one-sided couplings. Such fast and slow rotation rates of spiral waves, for these three types of couplings, can be understood by analyzing the AP morphology of a single MF composite and the CV of plane waves, with these three types of couplings. In general, our studies have shown that, if the fibroblasts in the MF composites act as current sources (current sinks), then the rate of rotation of the spiral wave increases (decreases).

Spiral waves in inhomogeneous domains. We now examine the effects of fibroblast inhomogeneities on spiral-wave dynamics in our mathematical model; outside the region of the inhomogeneity we use the TNNP model for cardiac tissue; inside the inhomogeneity we use the 2D MF composite domain that we have used in our studies above. We model an inhomogeneity in our 2D simulation domain by incorporating a small square patch of side $\ell$; this patch is an MF composite domain; the remaining part of the simulation domain contains only myocytes that are coupled via $D_{mm}$. Again, we focus on three different types of couplings in the MF-composite domain, namely, zero-, single-, and double-sided couplings between myocytes and fibroblasts; and we choose to the following,
representative fibroblast parameters: \( C_{F,\text{tot}} \approx 6.3 \) pF, \( G_F \approx 4 \) nS, and \( E_F \approx -39 \) mV. In most of our studies, we use \( G_{\text{gap}} \approx 8 \) nS.

In Figs. 10 (a), (b), and (c) we show pseudocolor plots of \( V_m \) at time, \( t \approx 2 \) s, in the presence of a square fibroblast inhomogeneity, of side 33.75 mm, for the case of zero-sided coupling, and the lower-left-hand corner of the inhomogeneity at, respectively, \( (x = 33.75 \text{ mm}, y = 67.5 \text{ mm}) \), \( (x = 56.25 \text{ mm}, y = 56.25 \text{ mm}) \), and \( (x = 78.75 \text{ mm}, y = 45 \text{ mm}) \); the white solid lines in these figures show the spiral-tip trajectories in the time interval \( 2s \leq t \leq 3s \) and the local time series data are recorded from points that are shown by asterisks. Video S6 illustrates the spatiotemporal evolution of these spiral waves. The plots in (d)-(f) show the time series for \( V_m \) in the interval \( 0s \leq t \leq 4s \), which are obtained from a point outside \( (x = 112.5 \text{ mm}, y = 112.5 \text{ mm}) \) for all cases and inside the fibroblast inhomogeneity \( (x = 45 \text{ mm}, y = 90 \text{ mm}), (x = 67.5 \text{ mm}, y = 67.5 \text{ mm}) \), and \( (x = 90 \text{ mm}, y = 67.5 \text{ mm}) \) for (a), (b), and (c), respectively, represented by black circles and red triangles, respectively; (g), (h), and (i) show plots of the inter-beat intervals (ibis) versus the beat number \( n \) for the time series of \( V_m \) mentioned above; each one of these time series contain \( 2 \times 10^5 \) data points; the power spectra \( E(f) \), which follow from these time series, are given in (j), (k), and (l).

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Figure 10. Pseudocolor plots of the transmembrane potential of the myocyte \( V_m \) at time, \( t \approx 2 \) s, in the presence of a square shape MF composite inhomogeneity, of side \( \ell = 33.75 \) mm, for the case of zero-sided coupling; the bottom-left corner of the inhomogeneity is fixed at (a) \( (x = 33.75 \text{ mm}, y = 67.5 \text{ mm}) \), (b) \( (x = 56.25 \text{ mm}, y = 56.25 \text{ mm}) \), and (c) \( (x = 78.75 \text{ mm}, y = 45 \text{ mm}) \); the white solid lines in these figures show the spiral-tip trajectories in the time interval \( 2s \leq t \leq 3s \) and the local time series data are recorded from points that are shown by asterisks. Video S6 illustrates the spatiotemporal evolution of these spiral waves. The plots in (d)-(f) show the time series for \( V_m \) in the interval \( 0s \leq t \leq 4s \), which are obtained from a point outside \( (x = 112.5 \text{ mm}, y = 112.5 \text{ mm}) \) for all cases and inside the fibroblast inhomogeneity \( (x = 45 \text{ mm}, y = 90 \text{ mm}), (x = 67.5 \text{ mm}, y = 67.5 \text{ mm}) \), and \( (x = 90 \text{ mm}, y = 67.5 \text{ mm}) \) for (a), (b), and (c), respectively, represented by black circles and red triangles, respectively; (g), (h), and (i) show plots of the inter-beat intervals (ibis) versus the beat number \( n \) for the time series of \( V_m \) mentioned above; each one of these time series contain \( 2 \times 10^5 \) data points; the power spectra \( E(f) \), which follow from these time series, are given in (j), (k), and (l).

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The white solid lines in Figs. 10 (a)–(c) show the trajectories of the spiral tips for \( 2s \leq t \leq 3s \); note that these trajectories depend sensitively on the position of the MF composite inhomogeneity; furthermore, the dynamics of the wave, inside and outside this inhomogeneity, are different (cf., our previous studies with ionic inhomogeneities [16,17]). Figure 10 (a) shows that, in the presence of an MF-composite inhomogeneity, the tip trajectory deviates from one with a circular core; the corresponding plots of the ibis
and power spectra, Figs. 10 (g) and (j), respectively, illustrate that the temporal evolution of the spiral is periodic inside and outside of the inhomogeneous domain. However, for the inhomogeneity of Fig. 10 (b), we observe non-periodic and periodic temporal evolutions, respectively, inside and outside the MF inhomogeneity as can be surmised from the ibi and power-spectra plots in Figs. 10 (h) and (k); the tip has a Z-type trajectory, extends over a length \( \approx 34 \) mm, and it meanders both inside and outside the inhomogeneity. For the inhomogeneity of Fig. 10 (c), we obtain periodic and non-periodic temporal evolutions, respectively, inside and outside the MF-composite inhomogeneity (see Figs. 10 (i) and (l) for the ibi and power spectra); here the tip trajectory has a linear extent \( \approx 14 \) mm and it is restricted, predominantly, inside the inhomogeneity. The Video S6 has four animations that show superimpositions of pseudocolor plots of \( V_m \) and the spiral-tip trajectories for \( 2 \leq t \leq 3 \) s for a control myocyte layer with no inhomogeneities and the simulation domains for Figs. 10 (a)–(c). If we use one-sided coupling with \( G_{mn}/G_{ff} = 1 \), we find that our results are similar to those we have depicted in Fig. 10 for zero-sided coupling, as we show in Fig. S7 in Material S1. In general, the heterogeneity of cardiac tissue causes a drift of the spiral wave towards regions in which a spiral wave has a long period of rotation [64–66]. In our studies, the period of a spiral wave decreases slightly inside the fibroblast heterogeneity; and the final position of the spiral depends on the location of the heterogeneity.

Next we investigate the dependence of spiral-wave dynamics on the size of the MF-composite inhomogeneity. Figures 11 (a), (b), and (c) show pseudocolor plots, at time \( t = 2 \) s, of \( V_m \) for three representative square inhomogeneities, with sides \( \ell = 22.5 \) mm, \( \ell = 33.75 \) mm, and \( \ell = 45 \) mm; the lower-left-hand corner of these squares is fixed at \( (56.25 \) mm,\( 56.25 \) mm). The white, solid lines in these figures show the spiral-tip trajectories for \( 2 \leq t \leq 3 \) s. The spiral-tip trajectory, shown in Fig. 11(a), is a closed, but not circular, path that is confined inside the inhomogeneity; as the size of the inhomogeneity grows, this tip trajectory also grows in size and part of it lie outside the inhomogeneity, as shown, e.g., in Fig. 11(b) and (c); in the latter two cases, the tip trajectories are not closed and their linear extent is comparable to the length of the side of the inhomogeneity. We also obtain time series for \( V_m \) from a point outside the inhomogeneity (\( 22.5 \) mm,\( 22.5 \) mm) and a point inside it (67.5 mm,67.5 mm); these points are indicated, respectively, by black and white asterisks in Figs. 11 (a), (b), and (c); and data from the points outside and inside the fibroblast inhomogeneity are represented, respectively, by black circles and red triangles in Figs. 11 (d)–(l). In Figs. 11 (d), (e), and (f) we give the time series of \( V_m \) for \( V_m \) and power spectra in Figs. 11 (g)–(l). Note, furthermore, that the spiral-wave rotation period \( \tau_{rot} \) decreases as the size of inhomogeneity increases; this is consistent with Fig. 9 (e), which shows that the ibi for a homogeneous myocyte layer is greater than the ibi of a homogeneous MF-composite layer.

We turn now to a study of an MF-composite inhomogeneity with double-sided coupling. We have shown that, in a homogeneous, 2D simulation domain with such MF composites, the occurrence of conduction block depends on the value of \( G_{mf} \); e.g., we have observed that conduction failure occurs if \( G_{mn}/G_{ff} \leq 4 \) and \( G_{gap} = 8 \) nS, but it does not occur if \( G_{gap} = 8 \) nS and \( G_{mn}/G_{mf} > 4 \); in the latter case, \( CV \) depends on the ratio \( G_{mn}/G_{mf} \). Therefore, we choose the following four representative values for \( G_{mf} \) in our MF-composite inhomogeneity studies: \( G_{mn}/G_{mf} = 1, G_{mn}/G_{mf} = 50, G_{mn}/G_{mf} = 100, \) and \( G_{mn}/G_{mf} = 200 \). We begin with \( G_{mn}/G_{mf} = 1 \) for which conduction failure occurs for all physical values \( G_{ff} \) and \( G_{gap} \) in the homogeneous case. In Figs. 12 (a)–(h), we show pseudocolor plots of \( V_m \) at time \( t = 2 \) ms, when a square MF-composite inhomogeneity, with \( G_{mn}/G_{ff} = 1 \) and \( G_{mn}/G_{mf} = 1 \), is placed with its lower-left corner at \( (x = 67.5 \) mm,\( y = 67.5 \) mm); the square has a side length \( \ell = 0 \), i.e., no inhomogeneity (Fig. 12(a)), \( \ell = 11.25 \) mm (Fig. 12(b)), \( \ell = 16.875 \) mm (Fig. 12(c)), \( \ell = 22.5 \) mm (Fig. 12(d)), \( \ell = 28.125 \) mm (Fig. 12(e)), \( \ell = 33.75 \) mm (Fig. 12(f)), \( \ell = 39.375 \) mm (Fig. 12(g)), and \( \ell = 45 \) mm (Fig. 12(h)). The smallest MF-composite inhomogeneity that can anchor a spiral wave has \( \ell = 16.875 \) mm. The Video S8 has four panels that show the spatiotemporal evolution of pseudocolor plots of \( V_m \) for \( 2 \leq t \leq 3 \) s for a control myocyte layer with no inhomogeneities (top left panel) and the simulation domains for Figs. 12 (d), (f), and (h) (top right, bottom left, and bottom right panels, respectively). Figure 12(i), a plot of the rotation period \( \tau_{rot} \) of such an anchored spiral wave, versus \( \ell \), shows how \( \tau_{rot} \) increases with \( \ell \); such an increase has also been seen for a conduction inhomogeneity [67].

If the value of \( G_{mn}/G_{mf} \) is such that conduction failure occurs in a homogeneous, MF-composite simulation domain, then the MF-composite inhomogeneity behaves somewhat like a conduction inhomogeneity inasmuch as the spiral wave does not enter significantly into the region of the inhomogeneity. We discuss in detail in Material S1 (Fig. S8) how far a spiral wave penetrates into an MF-composite inhomogeneity. We find that, depending on the values of parameters, the MF-composite inhomogeneity can act like a conduction inhomogeneity, or, the spiral wave can penetrate the region of the inhomogeneity marginally, i.e., the MF-composite inhomogeneity can act like an ionic inhomogeneity [16].

In our plane-wave studies in 2D homogeneous simulation domains with double-sided coupling, we have noted that the propagation speed \( CV \) and the wave length \( \lambda \) depend \( G_{mf} \). Therefore, we now carry out a study of the interaction of spiral waves with an MF-composite inhomogeneity for different values of \( G_{mf} \). We have seen above that an MF-composite inhomogeneity behaves somewhat like a conduction inhomogeneity if \( G_{mn}/G_{mf} = 1 \). In Figs. 13 (a), (b), and (c) we show, for \( G_{mn}/G_{mf} = 50, 100, \) and 200, respectively, pseudocolor plots of \( V_m \), at time \( t = 2 \) s, in the presence of a square, MF-composite inhomogeneity, of side \( \ell = 33.75 \) mm and with its lower-left-hand corner placed at \( (x = 56.25 \) mm,\( y = 56.25 \) mm) for the case of doubled-sided coupling with \( G_{mn}/G_{ff} = 1 \) and \( G_{gap} = 8 \) nS. We also obtain time series for \( V_m(x,y,t) \) from a point outside the inhomogeneity (\( x = 22.5 \) mm,\( y = 22.5 \) mm) and a point inside it (\( x = 67.5 \) mm,\( y = 67.5 \) mm), both of which are depicted by asterisks in Figs. 13 (a)–(c). These time series, with \( 2 \times 10^5 \) data points each, are plotted in Figs. 13 (d), (e), and (f) for \( G_{mn}/G_{mf} = 50, 100, \) and
200, respectively (data from the points outside and inside the inhomogeneity are represented, respectively, by black circles and red triangles); Figs. 13 (g), (h), and (i) show the corresponding plots of the inter-beat intervals (ibis) versus the beat number $n$; and the associated power spectra $E(\omega)$ are depicted in Figs. 13 (j), (k), and (l). The Video S9 has four panels that show the spatiotemporal evolution of pseudocolor plots of $V_m$ and the spiral-tip trajectories for $2s \leq t \leq 3s$ for a control myocyte layer with no inhomogeneities (top left panel) and, in addition, the simulation domains of Figs. 13 (a)-(c) (top right, bottom left, and bottom right panels). We observe rich spiral-wave dynamics, which can be different inside and outside of the MF-composite inhomogeneity, as in the cases with zero- and single-sided couplings.

Figure 11. Pseudocolor plots of $V_m$ with MF-composite inhomogeneities: Pseudocolor plots of the transmembrane potential of the myocyte $V_m$ at time, $t=2s$, in the presence of three square MF-composite inhomogeneities with sides (a) $\ell = 22.5\, \text{mm}$, (b) $\ell = 33.75\, \text{mm}$, and (c) $\ell = 45\, \text{mm}$, for the case of zero-sided coupling; the bottom-left corner of these squares is fixed at $(x = 56.25\, \text{mm}, y = 56.25\, \text{mm})$; the white solid lines in these figures show the spiral-tip trajectories in the time interval $2s \leq t \leq 3s$ and the local time series data are recorded from points that are shown by asterisks. Video S7 illustrates the spatiotemporal evolution of these spiral waves. The plots in (d)-(f) show the time series for $V_m$, in the interval $0s \leq t \leq 4s$, which are obtained from the point outside the inhomogeneity $(x = 22.5\, \text{mm}, y = 22.5\, \text{mm})$ and a point inside it $(x = 67.5\, \text{mm}, y = 67.5\, \text{mm})$, represented by black filled circles and red filled triangles, respectively; (g), (h), and (i) show plots of the inter-beat intervals (ibis) versus the beat number $n$ for the time series of $V_m$, mentioned above; each one of these time series contain $2 \times 10^6$ data points; the power spectra $E(\omega)$, which follow from these time series, are given in (j), (k), and (l).

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We examine the interaction of spiral waves with an MF-composite inhomogeneity for different values of $G_{gap}$ in detail in Material S1 (Fig. S9). The Video S10 has four panels that show the spatiotemporal evolution of pseudocolor plots of $V_m$ and the spiral-tip trajectories for $2s \leq t \leq 3s$ for a control myocyte layer with no inhomogeneities (top left panel) and, in addition, the simulation domains of Figs. S9(a)-(c) in Material S1 (top right, bottom left, and bottom right panels). Here too we obtain a rich variety of spiral-wave behaviors inside and outside of the MF-composite inhomogeneity, as in the cases with zero- and single-sided couplings.
3 Control of Spiral-wave Turbulence in the Presence of MF-composite Inhomogeneities

One of the goals of our extensive studies of various types of heterogeneities in mathematical models for cardiac tissue [16,17,68,69] has been to understand their effects on spiral-wave dynamics and thus develop effective, low-amplitude control techniques for the elimination of single, rotating spiral waves or spatiotemporally chaotic multiple spiral waves of electrical activation in mathematical models for cardiac tissue. In these earlier studies [16,17,68,69] we have considered conduction or ionic inhomogeneities; here we have extended such studies to mathematical models in which we allow for the MF-composite inhomogeneities that we have described above.

In this subsection we investigate the elimination of spiral-wave turbulence in the presence of MF-composite inhomogeneities. We use the control scheme of Sinha, et al., [19]; this eliminates spiral waves by the application of a current pulse on a mesh, which we describe below. We have found in our earlier studies [16–19] that such a mesh-based control scheme is effective even when the simulation domain has conduction or ionic inhomogeneities; by contrast, control schemes, which use electrical stimuli at a point [70,71], work well in homogeneous simulation domains but do not eliminate spiral-wave turbulence in domains with inhomogeneities.

In our mesh-based control scheme in a 2D simulation domain with an MF-composite inhomogeneity, we apply a current pulse of amplitude 30 pA/pF for 400 ms over a mesh that divides our square simulation domain, of side 135 mm, into 16 square cells of side 33.75 mm each. The application of this pulse makes the region, which is covered by the mesh, refractory and, therefore, effectively imposes Neumann boundary conditions for any cell bounded by this mesh. Thus, spiral waves that lie inside the cell are absorbed at the mesh that bounds it and, eventually, spiral-wave turbulence is eliminated from the whole simulation domain.

We begin with a discussion of the control of spiral waves, in a 2D, MF-composite simulation domain, by the application of a current pulse on the square mesh described above. In Fig. 14 (a) we show a pseudocolor plot of \( V_m \) at time \( t = 0 \) ms, for the control case with \( G_{gap} = 0 \) nS; we give pseudocolor plots of \( V_m \) at \( t = 400 \) ms, and in the absence and presence of the control pulse in Figs. 14 (b) and (c), respectively. Figures 14 (d), (e), and (f) are the analogs of Figs. 14 (a), (b), and (c), respectively, for zero-sided coupling with \( C_{f,tot} = 6.3 \) pF, \( G_f = 4 \) nS, \( E_f = -39 \) mV, and \( G_{gap} = 8 \) nS. Figures 14 (g), (h), and (i) are the analogs of Figs. 14 (a), (b), and (c), respectively, for two-sided coupling with \( C_{f,tot} = 6.3 \) pF, \( G_f = 4 \) nS, \( E_f = -39 \) mV, \( G_{gap} = 8 \) nS, \( G_{num}/G_{tot} = 1 \), and \( G_{num}/G_{tot} = 200 \). From the pseudocolor plots of \( V_m \) in Figs. 14 (c), (f), and (i) we see that our mesh-based, spiral-control scheme succeeds in eliminating spiral-wave turbulence in less than 400 ms in a 2D, MF-composite simulation domain with zero- and two-sided couplings; we have obtained similar results with one-sided coupling too. The Video S11, which comprises six animations of pseudocolor plots of \( V_m \), shows the spatiotemporal evolution of the spiral waves for these cases, with and without control pulses.

Figure 12. Pseudocolor plots of the transmembrane potential of the myocyte \( V_m \) at time, \( t = 2 \) s, when a square MF-composite inhomogeneity, with \( G_{num}/G_f = 1 \) and \( G_{num}/G_{tot} = 1 \), is placed with its lower-left corner at \((x = 67.5 \text{ mm}, y = 67.5 \text{ mm})\); the square has a side of length (a) \( l = 0 \), i.e., no inhomogeneity, (b) \( l = 11.25 \text{ mm} \), (c) \( l = 16.875 \text{ mm} \), (d) \( l = 22.5 \text{ mm} \), (e) \( l = 28.125 \text{ mm} \), (f) \( l = 33.75 \text{ mm} \), (g) \( l = 39.375 \text{ mm} \), and (h) \( l = 45 \text{ mm} \). Video S8 illustrates the spatiotemporal evolution of these spiral waves for (a), (d), (f) and (h). The smallest MF-composite inhomogeneity that can anchor a spiral wave has \( l = 16.875 \text{ mm} \); (i) shows a plot of the rotation period \( t_{rot} \) of such an anchored spiral wave.

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We now study spiral-wave control in a 2D simulation domain with myocytes and a square MF-composite inhomogeneity with side $l \approx 33.75$ mm whose bottom-left corner is placed at $(x \approx 56.25$ mm, $y \approx 56.25$ mm). Again, we apply a control pulse of amplitude $30 \text{ pA/pF}$ for $t = 400$ ms over a square mesh with cells whose sides are of length $L = K \approx 33.75$ mm, i.e., the simulation domain is divided into $4 \times 16$ square blocks. We consider the following three cases: (A) zero-sided coupling with $G_{\text{mm}}/G_{\text{ff}} = 1$ and $G_{\text{gap}} = 8 \text{ nS}$; (B) double-sided coupling with the same fibroblasts parameters as in case (A) and $G_{\text{mm}}/G_{\text{mm}} = G_{\text{ff}} = K$; and (C) double-sided coupling with the same fibroblasts parameters as in case (A) and $G_{\text{mm}}/G_{\text{mm}} = G_{\text{ff}} = 200$. Figures 15 (a), (d), and (g) show pseudocolor plots of $V_m$ at $t = 0$ for cases (A), (B), and (C), respectively. Figures 15 (b), (c), and (h) show their analogs for $t = 400$ ms when no control pulse is applied. Figures 15 (c), (f), and (i), which show pseudocolor plots of $V_m$ at $t = 0$ for cases (A), (B), and (C), respectively, at $t = 400$ ms when we apply a control pulse, illustrate how our control scheme is effective in suppressing spiral-wave turbulence in the presence of an MF-composite inhomogeneity. For the spatiotemporal evolution of these spiral waves see Video S12.

**Discussion and Conclusion**

We have carried out detailed numerical studies of an MF composite by modelling human ventricular myocyte cells, as in Ref. [15], and fibroblasts as passive RC circuits, as in Refs. [9,12]. The passive nature of these fibroblasts makes them behave as inexcitable cells and, therefore, they act either as current sources or sinks, when they are coupled with myocytes. We have...
investigated the responses of such MF composites to external electrical stimuli by varying systematically the total cellular capacitance $C_{f,tot}$ of fibroblasts and their membrane conductance $G_f$, resting membrane potential $E_f$, gap-junctional coupling (with myocytes) $G_{gap}$, and the number $N_f$ of fibroblasts coupled to a myocyte over a wide range of biophysically relevant values [3,5,46,49,50]. We find that the myocyte APD increases as we increase $C_{f,tot}$ in the fibroblast; but it does not significantly affect the shape of the AP. An increase in $G_f$ prolongs the APD, if the resting potential of the fibroblast is high ($E_f > -19$ mV), but it shortens the APD if $E_f < -19$ mV; and $V_{rest}$ is elevated substantially as $G_f$ is increased. We focus mainly on the dependence of the AP morphology on $E_f$ and $G_{gap}$, because the values of these two parameters span a wide range in experiments [3,5,49,50]. We find that the APD increases with $G_{gap}$ (Fig. 2), if $E_f$ is high, but it decreases, as $G_{gap}$ increases, if $E_f$ is low. The maximum upstroke velocity, $dV/dt_{max}$, and the maximum value of the myocyte transmembrane potential, $V_{max}$, decrease as we increase $E_f$ (Fig. 2), with a fixed value of $G_{gap}$, whereas the notch of the AP, $V_{notch}$, the maximum of the plateau, $V_{plateau}$, and the resting membrane potential all increase as we increase $E_f$. Furthermore, we have shown that $dV/dt_{max}$, $V_{max}$, $V_{notch}$, and $V_{plateau}$ decrease, but $V_{rest}$ increases, as we increase $G_{gap}$ (Fig. 2), with a fixed value of $E_f$. We have carried on simulations to check the dependence of the myocyte AP on the number $N_f$ of fibroblasts, which are coupled to a myocyte. We have observed that (a) the APD and $V_{rest}$ decrease and increase, respectively, as $N_f$ increases and (b) this increase of $V_{rest}$ depolarizes the membrane potential so that, eventually, $V_m$ crosses the threshold value for the generation of an AP, and, therefore, the MF composite cell begins to show either autorhythmic or oscillatory behaviors [57].

 Fibroblasts are found to be much smaller than myocytes in experimental studies [8,72]; thus, in cell-culture experiments, more than one fibroblast can be deposited per myocyte in the cell culture. Furthermore, the sizes of fibroblasts can vary in such experiments; this depends on the preparation technique and circumstances; and the fibroblast size decides the maximum number of fibroblasts that can attach to a myocyte. Indeed, a wide range of values has been used for the total cellular capacitance $C_{f,tot}$ in various computer models [9,11,12] because these models assume that $C_{f,tot}$ is related to the size of the fibroblast. Hence, in our model studies, for a given size of fibroblast, i.e., a fixed value of $C_{f,tot}$, the complex, network-type interaction can occur via $G_{eff}$, $G_{inf}$, and $G_{fms}$, depending on the number of fibroblasts $N_f$ coupled to a myocyte in an MF composite. Therefore, we use $N_f$ fibroblasts per a myocyte site in an MF composite to study the wave dynamics; each of these fibroblasts is coupled to a myocyte via $G_{gap}$. We think of this collection of $N_f$ fibroblasts as a single cluster that interacts via $G_{gap}$ and $G_{fms}$, with its neighboring myocytes; we assume that the number of fibroblast clusters is

Figure 14. Spiral-wave control in our 2D M-F composite model, by the application of a control pulse of amplitude 30 pA/pF for $t=400$ ms over a square mesh with each block of side $L = 33.75$ mm, i.e., the simulation domain is divided into $4^2$ square blocks. Plots in (a), (d) and (g) are the initial conditions of $V_m$, i.e., $t=0$ ms, for the control case, i.e., $G_{gap}=0$ nS, zero sided coupling with $C_{f,tot}=6.3$ pF, $G_f=4$ nS, $E_f=-39$ mV and $G_{gap}=8$ nS, and double-sided coupling with the same fibroblasts parameters as in (d) and with $G_{mm}/G_{fp}=1$ and $G_{nm}/G_{mf}=200$, respectively. (b), (e) and (h) show the pseudocolor plots of $V_m$, at $t=400$ ms in the absence of any control pulse. However, the spiral-wave is suppressed by an application of the control pulse as shown (c), (f) and (i) at time $t=400$ ms. The Video S11, which comprises six animations of pseudocolor plots of $V_m$, shows the spatiotemporal evolution of these spiral waves for these cases, with and without control pulses.

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Figure 15. Spiral-wave control in the 2D Fibroblast model in the presence of a square shape MF composite inhomogeneuty of size \( l = 33.75 \) mm whose bottom-left corner is placed at \((56.25 \) mm, \( 56.25 \) mm). We apply a control pulse of amplitude 30 pA/pF for \( t = 400 \) ms over a square mesh with each block of size \( l / K = 33.75 \) mm, i.e., the simulation domain is divided into \( 4^2 \) square blocks. Plots in Figs. (a), (b) and (c) are the initial conditions of \( V_m \), i.e., \( t = 0 \) ms, for zero sided coupling with \( C_{f,\text{tot}} = 6.3 \) pF, \( E_f = 4 \) nS, \( E_f = -39 \) mV and \( G_{gap} = 8 \) nS, double sided coupling with same fibroblasts parameters as in (a) with \( G_{mm}/G_{EF} = 1 \) and \( G_{mm}/G_{EF} = 1 \), and double sided coupling with same fibroblasts parameters as in (a) with \( G_{mm}/G_{EF} = 1 \) and \( G_{mm}/G_{EF} = 200 \), respectively. Figures (b), (e) and (h) are the time evolution of \( V_m \) corresponds to Figs. (a), (d), (g), respectively, at \( t = 400 \) ms in absence of any control pulse. However, the spiral-wave is suppressed an application of control pulse as shown in Figs. (c), (f) and (i) at time \( t = 400 \) ms. For the spatiotemporal evolution of these spiral waves see Video S12. doi:10.1371/journal.pone.0072950.g015

exactly equal to the number of myocyte cells. We assume that, at the level of a first approximation, the fibroblasts in a cluster interact with each other only to the extent that they form cluster. When the size of the fibroblast cluster is much smaller than the size of a myocyte, then the couplings \( G_{mf}, G_{fm}, \) and \( G_{ff} \) are irrelevant and we should only use a zero-sided model.

 Earlier computational studies [9–12], which we have discussed in the “Introduction” section, have not investigated the dependence of the AP morphology, as we do, on the parameters \( C_{f,\text{tot}}, G_f, E_f, \) \( G_{gap}, \) and \( N_f \); most of these earlier studies have focused on the dependence of the AP on \( N_f \), principally, and \( E_f \), to some extent. Our detailed studies of the dependence of the AP morphology on \( C_{f,\text{tot}}, G_f, E_f, \) \( G_{gap}, \) and \( N_f \) are designed to help experimentalists in the growth of different tissue layers with myocytes and fibroblasts and thus uncover the contribution of fibroblasts to the mechanisms of ventricular fibrillation. For example, in our simulation, we have found that \( E_f \) has the potential to alter the APD and, therefore, it can play a crucial role in spiral-wave dynamics.

We have performed extensive numerical simulations in two-dimensional (2D) simulation domains, both homogeneous and inhomogeneous, which contain myocytes or MF composites with zero-, one-, or two-sided couplings between myocytes and fibroblasts. We have found that, for zero- and one-sided couplings, the plane-wave conduction velocity \( CV \) decreases as \( G_{gap} \) increases, but, with two-sided couplings, \( CV \) first decreases very rapidly and then rises slowly as \( G_{gap} \) increases; furthermore, we have found that conduction failure can occur in the double-sided case if \( G_{mf} \) and \( G_{gap} \) are low (Fig. 6). Such behaviors have been seen in earlier numerical studies, with passive or active fibroblast in models [13,14] that are similar to, but not the same as, our mathematical model, and in cell cultures [13,24–26]. In our studies with zero-sided coupling, we have found that (a) the rotation period \( \tau_{\text{rot}} \) of a spiral wave decreases as we increase \( G_{gap} \) (Fig. 7), which we have explained qualitatively, (b) the higher the value of \( G_{gap} \), the more compact and closed is the trajectory of the spiral tip (this compactness prevents a single spiral from splitting into multiple spirals [73–75]), and (c) in the parameter range in which autorhythmicity occurs, the spiral wave rotates periodically, for low frequencies (Figs. S6 in Material S1). Our studies of spiral-wave dynamics in a homogeneous simulation domain with MF composites has shown that the spiral rotates faster for single-sided coupling than for zero-sided coupling. The spiral rotation rate in the double-sided case lies between these rates for zero- and one-sided couplings. We have presented a qualitative explanation of such fast and slow rotation rates of spiral waves by analyzing the AP morphology of a single MF composite and the plane-wave \( CV \) with these three types of couplings. In general, our studies have shown that, if the fibroblasts in the MF composites act as current sources (current
sinks), then the rate of rotation of the spiral wave increases (decreases).

Our studies of spiral-wave dynamics in the presence of localized, MF-composite inhomogeneities have shown that they may block spiral-wave propagation like conduction inhomogeneities [16,28,30,68,69,76]; such behavior is the consequence of conduction failure, which we have observed in our plane-wave studies in homogeneous domains. However, in some cases the spiral wave can enter the region with the MF-composite inhomogeneity and yield rich spiral-wave dynamics, which can be different inside and outside of the inhomogeneity, as happens, e.g., when we have ionic inhomogeneities [16,31,69]. We have found that zero- and single-sided couplings have the potential to behave like ionic inhomogeneities; but double-sided couplings have the potential to behave either like conducton- or ionic-type inhomogeneities (depending on the value of $G_{mf}$). Furthermore, spiral-wave dynamics, with all these three types of couplings, depends sensitively on the position and size of our MF composite inhomogeneities [16,17].

Our model has some limitations: it does not have any mechanosensitive currents [77], either for the myocytes or the fibroblast. These types of ionic currents have been obtained in experimental studies of fibroblasts in the sino-atrial node (SAN) and atria of certain mammals [36,46,48,77,78]. These mechanosensitive currents may affect spiral-wave propagation if there is electro-mechanical feedback [79,80]; our model excludes such electro-mechanical feedback. The purely electrical approach, which we adopt, has also been used by several other groups (see, e.g., Refs. [19,76,81–86]) with the understanding that the approach, with which we adopt, has also been used by several other groups (see, e.g., Ref. [87]). Furthermore, our model is based on passive fibroblasts rather than active fibroblasts; there are several reasons to use passive fibroblast rather than active ones, the main reason being that, so far, no experiments have identified the presence of ionic current in human, ventricular fibroblasts; and the presence of active fibroblast in human, ventricular fibroblasts continues to be a matter of debate. The expression of ionic currents in fibroblasts have been observed in animal species, either in the region of the SAN or the right atrium. In spite of these limitations of our model, our work provides the most comprehensive study; attempted so far, of (a) the response of MF composites to external electrical stimulation and (b) the propagation of spiral waves in MF-composite 2D simulation domains, which are designed to model cell-culture experiments of the sort presented in Refs. [13,26]. We do not attempt here to study diffuse fibrosis; this lies beyond the scope of this paper; it has been addressed in other simulation studies [40,57,80–90]. Our studies have been designed specifically to uncover the role of fibroblasts in spiral-wave dynamics in the the absence of other tissue heterogeneities; once the underlying contributions of fibroblasts to spiral-wave dynamics have been revealed, we can incorporate tissue anisotropy and diffuse fibrosis as, e.g., in Ref. [57].

One of our goals is to test our low-amplitude control scheme in our 2D MF-composite model; we have focused here on a monodomain model, because we use low-amplitude control pulses rather than high-amplitude ones; the latter may require a bidomain model. Furthermore, some studies [91] have shown that there are no significant qualitative differences between bidomain and monodomain models, so we expect that our principal qualitative result will continue to hold even when such models are considered; this will have to be checked explicitly by subsequent studies.

### Supporting Information

#### Material S1 Supplementary Material (PDF)

#### Video S1 Plane-wave dynamics in our 2D, homogeneous myocyte-fibroblast (MF) model for different cases. (a) the control case, i.e., with only myocytes; (b) zero-sided coupling; (c) single-sided coupling with $G_{mn}/G_{ff} = 1$; (d) double-sided coupling with $G_{mn}/G_{ff} = 1$, $G_{mn}/G_{mf} = 100$, and $G_{gap} = 0.5$ nS; (e) double-sided coupling with $G_{mn}/G_{ff} = 1$, $G_{mn}/G_{mf} = 100$, and $G_{gap} = 8.0$ nS; and (f) double-sided coupling with $G_{mn}/G_{ff} = 1$, $G_{mn}/G_{mf} = 200$, and $G_{gap} = 0.5$ nS.

The time evolution of pseudocolor plots of the myocyte transmembrane potential $V_m$ is shown for $0s \leq t \leq 1s$; we use 10 frames per second (fps); in real time each frame is separated from the succeeding frame by 8 ms.

#### Video S2 Spiral-wave dynamics in our 2D homogeneous myocyte-fibroblast model with zero-sided coupling. (a) $G_{gap} = 0.00$ nS (control case, i.e., with only myocytes); (b) $G_{gap} = 0.50$ nS (low coupling); (c) $G_{gap} = 2.00$ nS (intermediate coupling); (d) $G_{gap} = 8.00$ nS (high coupling). Here spiral-tip trajectories, for $2s \leq t \leq 3s$, are shown by the white lines; we use 10 frames per second (fps); in real time each frame is separated from the succeeding frame by 8 ms.

#### Video S3 Spiral-wave dynamics in our 2D homogeneous myocyte-fibroblast model with zero-sided coupling. (a) $G_{gap} = 0.00$ nS (control case, i.e., with only myocytes); (b) $G_{gap} = 0.50$ nS (low coupling); (c) $G_{gap} = 2.00$ nS (intermediate coupling); (d) $G_{gap} = 8.00$ nS (high coupling). Here spiral-tip trajectories, for $4s \leq t \leq 5s$, are shown by the white lines; we use 10 frames per second (fps); in real time each frame is separated from the succeeding frame by 8 ms.

#### Video S4 Spiral-wave dynamics in our 2D homogeneous myocyte-fibroblast model with zero-sided coupling. (a) $G_{gap} = 0.00$ nS (control case, i.e., with only myocytes); (b) $G_{gap} = 0.50$ nS (low coupling); (c) $G_{gap} = 2.00$ nS (intermediate coupling); (d) $G_{gap} = 8.00$ nS (high coupling). Here spiral-tip trajectories, for $6s \leq t \leq 7s$, are shown by the white lines; we use 10 frames per second (fps); in real time each frame is separated from the succeeding frame by 8 ms.

#### Video S5 Spiral-wave dynamics in our 2D homogeneous myocyte-fibroblast model with gap-junctional conductance, $G_{gap} = 8.0$ nS. (a) control case; (b) zero-sided coupling; (c) single-sided coupling; (d) double-sided coupling. Here spiral-tip trajectories, for $2s \leq t \leq 3s$, are shown by the white lines; we use 10 frames per second (fps); in real time each frame is separated from the succeeding frame by 8 ms.

#### Video S6 Spiral-wave dynamics in our 2D myocyte-fibroblast model in the presence of a square fibroblast inhomogeneity, of side $\ell = 33.75$ mm, for the case of zero-sided, and the lower left-hand corner of the inhomogeneity at different positions. (a) control case; (b) ($x = 33.75$ mm, $y = 67.5$ mm); (c) ($x = 56.25$ mm, $y = 56.25$ mm); (d) ($x = 78.75$ mm, $y = 45$ mm). Here spiral-tip trajectories, for $2s \leq t \leq 3s$, are shown by the white lines; we use 10 frames per second (fps); in real time each frame is separated from the succeeding frame by 8 ms.
we use 10 frames per second (fps); in real time each frame is separated from the succeeding frame by 8 ms.

Video S9 Spiral-wave dynamics in our 2D MF-composite inhomogeneity model in the presence of a square MF-composite inhomogeneity of side \( \ell \) and with the lower-left-hand corner placed at \((x = 56.25 \text{ mm}, y = 56.25 \text{ mm})\). (a) control case; (b) \( \ell = 22.5 \text{ mm} \); (c) \( \ell = 33.75 \text{ mm} \); (d) \( \ell = 45 \text{ mm} \). Here spiral-tip trajectories, for \( 2s \leq t \leq 3s \), are shown by the white lines; we use 10 frames per second (fps); in real time each frame is separated from the succeeding frame by 8 ms.

Video S10 Spiral-wave dynamics in our 2D MF-composite inhomogeneity model in the presence of a square, MF-composite inhomogeneity, of side \( \ell = 33.75 \text{ mm} \) and with its lower-left-hand corner placed at \((x = 56.25 \text{ mm}, y = 56.25 \text{ mm})\) for the case of double-sided coupling with \( \frac{G_{\text{gap}}}{G_{\text{ff}}} = 1 \) and \( G_{\text{gap}} = 8 \text{ nS} \). (a) control case, i.e., with only myocytes; (b) \( \frac{G_{\text{gap}}}{G_{\text{ff}}} = 0.5 \text{ nS} \) (low coupling); (c) \( G_{\text{gap}} = 2.0 \text{ nS} \) (intermediate coupling); (d) \( G_{\text{gap}} = 8.0 \text{ nS} \) (high coupling). Here the myocyte transmembrane potential time evolution is shown for \( 2s \leq t \leq 3s \); we use 10 frames per second (fps); in real time each frame is separated from the succeeding frame by 8 ms.

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Author Contributions

Conceived and designed the experiments: ARN RP. Performed the experiments: ARN. Analyzed the data: ARN AVG RP. Contributed reagents/materials/analysis tools: ARN TKS AVG RP. Wrote the paper: ARN RP.