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The Cardiac Fibroblast: Functional and Electrophysiological Considerations in Healthy and Diseased Hearts

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Abstract

Cardiac fibrosis occurs in a number of cardiovascular diseases associated with a high incidence of arrhythmias. A critical event in the development of fibrosis is the transformation of fibroblasts into an active phenotype or myofibroblast. This transformation results in functional changes including increased proliferation and changes in the release of signaling molecules and extracellular matrix deposition. Traditionally fibroblasts have been considered to affect cardiac electrophysiology indirectly by physically isolating myocytes and creating conduction barriers. There is now increasing evidence that cardiac fibroblasts may play a direct role in modulating the electrophysiological substrate in diseased hearts. The purpose of this review is to summarize the functional changes associated with fibroblast activation, the membrane currents that have been identified in adult cardiac fibroblasts and describe recent studies of fibroblast-myocyte electrical interactions with emphasis on the changes that occur with cardiac injury. Further analysis of fibroblast membrane electrophysiology and their interactions with myocytes will lead to a more complete understanding of the arrhythmic substrate. These studies have the potential to generate new therapeutic approaches for the prevention of arrhythmias associated with cardiac fibrosis.

Keywords

Cardiac Fibroblast; Electrophysiology; Connexin; Ion Currents; Myofibroblast

Introduction

Cardiac electrophysiological research has been traditionally devoted to understanding the signaling processes and remodeling events that occur within the myocyte population in response to heart disease. Recently, it has been recognized that the non-myocyte cell populations in the heart, in particular cardiac fibroblasts, play an important role in its response to physiological and pathological stimuli. In addition, it is becoming increasingly clear that these cells may actively contribute to the electrophysiological remodeling that leads to the development of cardiac arrhythmias. Therefore, discerning the

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electrophysiological phenotype of cardiac fibroblasts and their response to injury is of paramount importance to understanding how they modulate the arrhythmogenic substrate. Ultimately, this may lead to novel antiarrhythmic therapeutic approaches targeted at the cardiac fibroblast population. This review focuses on our current knowledge of the electrophysiological phenotype of adult cardiac fibroblasts and their interactions with cardiac myocytes with emphasis on the functional changes that occur with injury.

The Cardiac Fibroblast

Fibroblasts are commonly found in connective tissues throughout the body. The relative number of fibroblasts in the heart varies between species. In the mouse heart the number of non-myocytes is approximately 45%[1] while in the rat heart non-myocytes account for approximately 70% of the cells.[1–3] During embryonic development, cardiac fibroblasts principally originate from mesenchymal cells of the proepicardium.[4,5] These cells migrate over the surface of the heart to form the epicardium which in turn gives rise to epicardial derived cells. The epicardial derived cells differentiate to form fibroblasts. During development the epicardial derived cells can alternate between fibroblasts and an α-smooth muscle actin (α-SMA) expressing myofibroblast phenotype.[4] Ultimately, most these cells assume the fibroblast phenotype, however, they can be reactivated to the myofibroblast phenotype in response to injury. In the postnatal heart, fibroblasts originate from endogenous cell populations, epithelial-to-mesenchymal transformation[6] and circulating bone marrow derived cells of hematopoietic origin.[7]

In the adult heart, cardiac fibroblasts express vimentin, β integrin and are mostly responsible for the secretion of fibronectin, laminin and collagen type I, III and IV.[8,9] To date, a fibroblasts specific marker has not been identified, however some discriminative markers exist. For example, the cell surface collagen receptor discoidin domain 2 was shown to be expressed in cardiac fibroblasts and absent in myocytes, vascular smooth muscle cells and endothelial cells.[10] Fibroblast specific protein-1 has also been proposed as a potential marker of fibroblast, however it has also been shown to be expressed in other cell types including monocytes, ischemic cardiomyocytes, endothelial cells and numerous cancer cells[11] [12,13]

Fibroblasts are phenotypically heterogeneous depending on developmental stage, organ and physiological conditions.[14–20] In the heart, atrial and ventricular fibroblasts have different cell densities in intact tissue.[17] Isolated cardiac fibroblasts have also been shown to have chamber specific differences with respect to morphology and proliferative responses to stimulation with growth factors.[17] Sheets and strands of fibroblasts are arranged in a 3D network that surrounds myocytes and other cell types.[10] This arrangement maintains continuity in different layers of the myocardial wall. Normal adult cardiac fibroblasts are flat and spindle shaped cells with multiple processes that form a network of cells within the extracellular matrix.[21] These interconnections allow fibroblasts to respond to a range of stimuli as well as giving them the potential to modulate function of myocytes as well as other cell types in the heart. In addition, fibroblasts have no contractile microfilaments or stress fibers.[22]

The coordinated action of fibroblasts involve secretion of a variety of signaling molecules, cytokines and growth factors.[21] Fibroblasts are responsible for maintenance of the extracellular matrix (ECM).[23] This process involves the highly regulated activity of collagen synthesis and degradation. Collagen synthesis is promoted by profibrogenic growth factors, including the transforming growth factor-β1 (TGF-β1) and degradation is accomplished by members of the matrix metalloproteinase (MMP) superfamily.[24]

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The Cardiac Myofibroblast

A critical event in the development of cardiac fibrosis is the transformation of fibroblasts into an active fibroblast phenotype or myofibroblast.[24] Transformation to the myofibroblast phenotype is strongly promoted by several cytokines and growth factors including TGF-β1.[25] Myofibroblasts, which are not present in normal cardiac tissue with the exception of the valve leaflets, express vimentin, α–SMA, collagen types I, III, IV and VIII, and have morphological and biochemical features intermediate between those of fibroblasts and smooth muscle cells.[26–28] These cells also express contractile proteins which are important in wound healing. The ultrastructural characteristics of myofibroblasts include myofilaments, well-developed rough endoplasmic reticulum and extensive cell-matrix contacts which are not observed in normal tissue.[29–31] Myofibroblasts are characterized by having increased rates of cellular proliferation as well as increased levels of ECM deposition. During wound healing these cells provide additional extracellular collagen fiber deposition which strengthens injured tissue. In most organs, myofibroblasts undergo apoptosis and disappear following the completion of tissue repair, while in the heart myofibroblasts have been shown to persist in mature infarct scars.[32–34]

The exact functional changes that occur as a consequence of cardiac fibroblast activation are beginning to be understood. Recent studies have highlighted important differences between fibroblasts isolated from normal and fibrotic hearts maintained under similar culture conditions. Figure 1 summarizes the functional consequences of cardiac fibroblasts activation which include altered proliferation,[17, 18, 35] migration,[18, 19] release of signaling molecules,[36] ECM deposition,[18–20] adhesion,[18, 19] and receptor expression.[20] Importantly, fibroblast activation has recently been associated with significant electrophysiological changes.[36, 37]

Squires et al[18] evaluated proliferation, migration, adhesion and collagen synthesis using fibroblasts isolated from infarcted and non-infarcted regions of left anterior descending coronary artery ligated mouse hearts. The same parameters were determined for fibroblasts isolated from normal hearts. Proliferation of fibroblasts isolated from the infarct region was increased 182% compared to normal hearts. Migration and adhesion to laminin were significantly decreased while adhesion to collagen IV and collagen synthesis were increased in fibroblasts isolated from the infarct region. Fibroblasts obtained from non-infarcted regions showed similar changes in migration, adhesion and collagen synthesis. Similar increases in proliferation of ventricular fibroblasts (vimentin positive cells) have been observed in intact tissue samples in response to aortic constriction.[35] Flack et al[19] studied the phenotypic changes of fibroblasts in response to injury using a porcine model of pacing induced heart failure. Left ventricular fibroblasts isolated from failing hearts showed increased migration, adhesion to collagen I, laminin and fibronectin. In addition, β1 integrin density, fibrillary collagen and MMP-2 were increased in fibroblasts isolated from failing hearts. Proliferation rates in this study were not found to be altered in fibroblasts isolated from failing hearts. However in a different study, proliferation rates were found to be increased in atrial and ventricular fibroblasts isolated from a canine tachypacing model of congestive heart failure.[17] The same study also showed an increase of platelet derived growth factor (PDGF) levels in response to tachypacing in fibroblasts isolated from atria but not ventricles. Similarly, another study by Jarvis et al[20] using fibroblasts obtained from infarcted and non-infarcted ovine myocardium showed that the characteristics of cardiac fibroblasts in culture varied depending on the source. Their findings demonstrated lower levels of α-SMA, increased collagen I, higher expression levels of natriuretic peptide receptors (NPR-A and NPR-B) and differences in the response to TGF-β1 and PDGF treatment between cells obtained from infarcted and non-infarcted hearts. In addition, several of these studies demonstrated that phenotypic differences between fibroblasts

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isolated from normal and diseased hearts were maintained in culture for several passages.[18–20] These data suggest that the activation process due to culture conditions does not fully replicate the *in vivo* activation process. Together these studies demonstrate fibroblast activation significantly alters the biological properties of cardiac fibroblasts and highlight the activation process as a potential therapeutic target.

Fibroblasts isolated from normal hearts and grown under standard tissue culture conditions begin expressing the myofibroblast marker α–SMA shortly after isolation.[38] These α–SMA positive fibroblasts have been referred to as myofibroblasts.[39–42] However, it is important to distinguish these *in vitro* differentiated cells from those obtained from fibrotic tissue. As we have discussed above, there is significant evidence suggesting *in vitro* differentiation does not fully replicate the *in vivo* activation process associated with cardiac fibrosis.

Fibroblast Membrane Currents

Although fibroblasts are considered non-excitable cells, significant interest in their electrophysiological properties has recently emerged. It is known that fibroblasts have a more depolarized resting membrane potential compared to myocytes.[43] However, there are are significant gaps in our understanding of fibroblast membrane ionic conductances. In particular, these cells have been recognized to have a high degree of heterogeneity yet ion channel expression in different heart chambers and under various physiological and pathophysiological conditions has not been systematically investigated. Recognizing this deficiency in our understanding of fibroblast electrophysiology and their potential role in cardiac arrhythmias, in this section we have organized the available literature based on heart chamber, recording conditions and whether the fibroblasts were obtained from normal or diseased tissue. Table 1 summarizes the ionic conductances that have been identified in adult atrial and ventricular fibroblasts.

Acutely Isolated Atrial Fibroblasts

Membrane electrophysiologic studies using acutely isolated adult atrial fibroblasts from normal hearts have been limited to the identification of a mechano-sensitive non-selective cation conductance in acutely isolated rat atrial fibroblasts.[44] Activation of this current was shown to result in depolarization or hyperpolarization of the resting membrane potential depending on whether cells were compressed or stretched. These findings are consistent with earlier observations using intact tissue.[45, 46] These studies have suggested that fibroblasts may play a role in mechano-electrical feedback in the intact heart.

Acutely Isolated Ventricular Fibroblasts

More information is available regarding membrane currents expressed in acutely isolated ventricular fibroblasts from normal hearts. Approximately 70% of freshly isolated adult rat ventricular fibroblasts from normal animals have been shown to express the inward rectifier current, I_{K1}, that is able to modulate membrane potential and respond to changes in the external potassium concentration.[47] These cells have also been shown to express a time and voltage dependent outward K^+ current.[47, 48] In addition to these K^+ conductances, freshly isolated ventricular fibroblasts express non-selective cation channels which are activated in response to NPR-C agonists.[49] These channels mediate the electrophysiological effects of C-type natriuretic peptides, may play a role in intracellular sodium and calcium levels and secretory properties of cardiac fibroblasts. Pharmacological and transcript analysis suggested that this current is mediated by several canonical transient receptor potential (TRP) channels including TRPC2, TRPC3 and TRPC5. Transcripts for other non-selective TRP cation channels including vanilloid-type (TRPV2, TRPV6) and melastatin related (TRPM4, TRPM8) are also expressed.

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Cultured Ventricular Fibroblasts

Current knowledge of membrane currents expressed in cultured adult ventricular fibroblasts is limited. Voltage dependent outward K^+ currents have been identified in cultured fibroblasts at similar current densities as freshly isolated cells.47 Transcript analysis suggested that Kv1.6 is responsible for this current. Recent studies have shown these cells express the vanilloid-type TRPV4 channel.50 This channel is highly responsive to environmental stimuli including heat and osmolarity. Activation of TRPV4 channels was shown to increase intracellular Ca^{2+} levels.50 In addition, transcripts for TRPV2 were also found. In cultured normal human cardiac fibroblasts functional ionic currents include a large conductance Ca^{2+} activated K^+ current,51, 52 delayed rectifier K^+ current, transient outward K^+ current, inward rectified K^+ current, Cl^- current, and a voltage gated Na^+ current.[52] The expression of many of these currents was shown to be highly heterogeneous.

Other studies have demonstrated membrane ionic conductances of ventricular fibroblasts undergo significant changes with time in culture.39, 47 Chilton et al demonstrated I_{K1} is present in a higher percentage of cultured compared to freshly isolated fibroblasts.47 In addition, I_{K1} density in cultured ventricular fibroblasts increases resulting in a shift of the reversal potential to more negative values. Transcript analysis suggested this current is primarily mediated by Kir2.1.47 Recent patch clamp and molecular studies have demonstrated functional expression of the K_{ATP} channel occurs with increasing time in culture.39 Benamer et al39 studied the electrophysiologial properties of fibroblasts using cells isolated from adult mice and maintained in culture for several days. The results showed functional expression of a glibenclamide-sensitive K^+ current which was linked to the activity of a channel composed of Kir6.1 and sulfonylurea receptor (SUR2) subunits. A summary of these findings is shown in Figure 2. The Western blots shown in Figure 2A demonstrate a progressive increase in Kir6.1 and SUR2 protein expression with time in culture. To evaluate the SUR2/Kir6.1 channel functional expression, the pinacidil-induced and the glibenclamide-sensitive whole cell currents were measured at 5, 7 and 9 days of culture. Figure 2B shows pinacidil induced a negligible glibenclamide-sensitive current in fibroblasts cultured for 5 days. After 7 days of culture, pinacidil activated a glibenclamide-sensitive current. Figure 2C shows fibronectin and α-SMA expression in cultured fibroblasts at different times in culture. α-SMA was absent at day 5, but progressively increased with time in culture. These results demonstrate that Kir6.1/SUR2 channel function appears progressively over time in culture and is associated with the *in vitro* differentiation of fibroblasts.

Atrial and Ventricular Fibroblasts from Diseased Hearts

Few studies have characterized the ionic conductances of fibroblasts obtained from diseased hearts. Cultured atrial fibroblasts isolated from human patients undergoing heart surgery for valvular or coronary artery disease express a voltage activated proton current.53 Acidification resulted in a decrease in outward current density which may be important for the response to ischemic events.

A different study demonstrated the expression of a non-selective cation current which is mediated by TRPM7 in freshly isolated human atrial fibroblasts from patients undergoing cardiac surgery.54 Right atrial fibroblasts were isolated from patients in normal sinus rhythm and atrial fibrillation (AF). TRPM7 current and Ca^{2+} influx were increased in AF patients compared to those in sinus rhythm. Treatment with TRPM7-specific shRNAs suggested this channel is responsible for Ca^{2+} influx in atrial fibroblasts from AF patients. TRPV2, TRPV4 and TRPC6-like currents could not be elicited in atrial fibroblasts even though their expression was detected by RT-PCR. In addition, a higher percentage of isolated atrial fibroblasts were α-SMA positive in AF compared to sinus rhythm patients.

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The percentage of α-SMA positive fibroblasts isolated from AF patients was reduced with TRPM7-specific shRNA treatment.

Finally, limited information is currently available on how fibroblast membrane conductance is affected with cardiac injury. A negative shift in the reversal potential and a significant increase in outward current density were observed in whole cell recordings from cultured ventricular fibroblasts isolated from infarcted compared to normal hearts.[36] Using microelectrodes in intact tissue, it has also been shown that the resting membrane potential shifts to more negative values and the membrane resistance increases in atrial fibroblasts from chronically infarcted rat hearts compared to normal hearts.[37] The magnitude of the membrane potential shift was shown to be dependent on infarct size. Interestingly, these findings are similar to the shift in reversal potential described for cultured compared to freshly isolated fibroblasts.[47]

Electrical Interactions Between Fibroblasts and Myocytes

Functional electrical coupling between myocytes and fibroblasts in the heart is currently a subject of substantial debate. Functional proof of myocyte-fibroblast coupling in the intact heart has been difficult to obtain using standard electrophysiological techniques. However, empirical evidence of propagation across cardiac transplant scars suggests *in vivo* electrical coupling between fibroblasts and/or myofibroblasts and myocytes.[55–62] In addition, ventricular fibroblasts express both connexin (Cx)43 and Cx45.[10] Cx43 has been shown to be localized at homocellular and heterocellular points of contact, while Cx45 is mainly present in fibroblasts and occasionally found between myocytes and fibroblasts. Electron microscopy studies of gap junction expression in sinoatrial node (SAN) tissue have shown that fibroblasts are regularly coupled by small gap junctions.[63] In addition, immunostaining and dye scrape loading experiments have shown that fibroblasts in the SAN are coupled via Cx40 in areas devoid of myocytes, and by Cx45 in regions where fibroblasts interact with myocytes.[64, 65]

Fibroblast connexin expression has also been evaluated in sheep ventricular infarct tissue. The labeling technique used in these studies did not allow for differentiation between fibroblasts and myofibroblasts.[66] Vimentin positive cells in the infarct scar express Cx43 or Cx45 with spatially and temporally distinct patterns.[66] Cx40 has not been identified in these cells. Fibroblasts expressing Cx45 infiltrate damaged tissue within the first few hours after infarction, reach their peak density within 6 days and decrease thereafter. The number of Cx43 expressing fibroblasts starts increasing 6 days after infarction, and continues to rise until at least the fourth week. These data suggest that Cx45 may be responsible for electrical coupling between fibroblasts and myocytes during the acute remodeling process, while Cx43 may be involved at later stages. It is currently unknown whether these cells represent a single fibroblast population that initially expresses Cx45 and then Cx43, or if there are two distinct populations. The latter is supported by evidence showing Cx43 and Cx45 do not colocalize. It is currently unknown if cardiac fibroblasts and myofibroblasts differ in their expression of connexins or gap junctions between themselves or with myocytes *in vivo*.

Several studies have demonstrated myocytes and fibroblasts in culture are able to establish electrical communication through gap junctions.[43, 67–71] Electrical interactions between myocytes and fibroblasts were initially described in cells isolated from neonatal hearts[43, 67, 70–72] and most studies have continued to utilize fibroblasts from neonatal sources.[41, 42, 73] Freshly isolated and cultured neonatal rat ventricular myocytes and neonatal fibroblasts readily form functional gap junctions, with single channel conductances of 43 pS between myocytes, 29 pS between myocytes and fibroblasts, and 22 pS between fibroblasts.[43, 71] Gap junctional coupling between myocytes and neonatal fibroblasts in culture

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supports the synchronization of contraction among individual myocytes,[69, 70] which are accompanied by synchronous membrane potential fluctuations in the interconnecting fibroblasts.[43, 67, 70] This fibroblast-mediated synchronization of myocytes is also supported by the high fibroblast membrane resistance that makes these cells good conductors.[74] Experiments using optical mapping techniques have shown that neonatal fibroblasts serve as sinks for electrotonic current, thereby producing localized slow conduction and decreases in the maximum rate of change of the action potential in the surrounding myocytes.[75] The use of transfected fibroblasts in these heterocellular cultures can significantly alter the conduction properties of the monolayer, which can be interpreted as evidence of electrical communication between the two cell types.[76] In addition, fibroblasts have been shown to modulate ectopic activity of myocyte monolayers.[40, 77] Other studies have demonstrated functional intercellular coupling between isolated adult myocytes and fibroblasts using dye transfer methods.[68, 78] Both Cx43 and Cx45 have been found in contact areas between neighboring fibroblasts and myocytes in culture.[41, 43, 68, 71, 73, 78, 79]

Despite the fact that there is evidence of myocyte-fibroblast electrical coupling *in vitro* dating back to the 1960s, the notion that interconnections between fibroblasts and myocytes allows electrotonic activation of cardiac tissue over extended distances was not addressed until recently. Gaudesius et al used a heterocellular culture model consisting of strands of neonatal rat myocytes interrupted by neonatal cardiac fibroblasts over defined distances to study fibroblast-mediated propagation.[73] Optical mapping measurements showed that propagation across a fibroblast bridge was possible over distances up to 300 μm, with significant conduction delays and slow conduction velocities in the bridge area. Interchanging fibroblasts with other non-excitable cell types expressing Cx43 increased the length threshold for successful conduction.

Until recently all electrophysiological studies investigating intercellular coupling between fibroblasts and myocytes had been performed using cells isolated from normal hearts. Vasquez et al[36] was the first to investigate connexin expression and functional coupling between myocytes and cardiac fibroblasts isolated from infarcted rat ventricles. A summary of the main findings of this study is shown in Figure 3. Figure 3A shows a Western blot demonstrating an increase in Cx43 levels in cultured fibroblasts obtained from infarcted compared to normal hearts. On average Cx43 protein levels were increased by 134%. Other studies have shown similar changes in protein levels in fibroblasts isolated from infarcted mouse hearts.[80] In addition Vasquez et al[36] demonstrated an increase in functional coupling between fibroblasts obtained from infarcted hearts and myocytes using Gap-FRAP. This technique consists of loading cultured cells with a gap junction permeable dye. The fluorescent molecules within a single cell are irreversibly photobleached using a high powered laser to create a concentration gradient with the surrounding cells. The recovery of fluorescence intensity in the bleached cell was then measured over time. The kinetics of the recovery of fluorescence are related to the degree of coupling between the bleached cell and the surrounding cells. Gap-FRAP experiments were performed using cultures of myocytes plated at a low density on top of confluent fibroblast monolayers obtained from normal or infarcted hearts. This plating configuration was selected to allow for recording of fluorescence recovery from myocytes. Figure 3B shows permeability constants obtained for myocytes plated on top of normal and infarcted fibroblasts. Recovery was 81% faster when myocytes were plated on top of fibroblasts obtained from infarcted hearts. Addition of the gap junction uncoupler carbenoxolone decreased the permeability constant confirming the fluorescence recovery was mediated by gap junctions. Myocyte electrophysiological parameters were evaluated in heterocellular cultures using high resolution optical mapping techniques. Fibroblasts from normal and infarcted hearts were plated at three different densities on top of confluent neonatal myocyte monolayers. Figure 3C–J shows

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representative activation maps, average CV and average APD_{70} values from the heterocellular cultures. Conduction velocities in cultures with fibroblasts from infarcted hearts were different from cultures with fibroblasts from normal hearts at all plating densities. In addition, APD_{70} was reduced in cultures with fibroblasts from infarcted compared to normal hearts at the lowest plating density. These data demonstrate cardiac injury results in important changes in the electrophysiological properties of fibroblasts that significantly alter fibroblast-myocyte interactions. These changes could alter the electrophysiological substrate and contribute to the formation of cardiac arrhythmias.

Electrical interactions between myocytes and fibroblasts have also been investigated using mathematical models.[81–89] Fibroblasts can affect conduction by acting as obstacles, creating electrotonic loading and depolarizing myocytes. Increase in myocyte resting membrane potential by coupling to the more depolarized fibroblasts can result in increased conduction velocity at low fibroblast densities.[86, 89] Further increases in fibroblast numbers slows down conduction and can eventually lead to conduction failure.[83, 84, 86, 88, 89] Myocyte-fibroblast coupling can also enhance conduction by allowing electrotonic conduction of the electrical activation between uncoupled myocytes. In addition, the effect of fibroblasts on conduction depends on the degree of intercellular coupling to myocytes. Similar to the effects observed at low fibroblast numbers, weak intercellular coupling between myocytes and fibroblasts results in a slight increase in myocyte resting membrane potential and conduction velocity.[89] At intermediate coupling levels, fibroblasts act as current sinks, resulting in slowing of conduction velocity and decreased maximum upstroke velocity.[83, 84, 88, 89] These effects become more pronounced as fibroblast numbers increase.

Coupling to myocytes can also modulate APD. APD values can increase or decrease depending on fibroblast resting membrane potential, fibroblast densities and the degree of coupling to myocytes.[82, 84, 87, 89] MacCannell et al. showed that coupling to fibroblasts can affect the rapid component of the delayed rectifier K^+ current, peak inward L-type Ca^{2+} current and peak inward Na^+/Ca^{2+} exchanger current in myocytes.[82] In addition to these changes, the increase in resting membrane potential due to coupling with fibroblasts can slow Na^+ channel recovery and extend post repolarization refractoriness.[86] These changes would facilitate induction of reentry with premature stimulation. In addition to the effects on APD, coupling to fibroblasts affects intracellular calcium cycling which can give rise to calcium alternans at the cellular and tissue scales.[87] Recent studies have also indicated that coupling with fibroblasts can increase the effects of pathophysiological stress in myocytes leading to an increase in EAD formation and repolarization failure.[85] In summary, modeling studies have shown electrical interactions between myocytes and fibroblasts can lead to complex effects on myocyte electrophysiology and conduction properties which combined with heterogeneous tissue structures may facilitate initiation and maintenance of arrhythmic activity.

Summary/Conclusions

Fibroblasts represent a major population of cells in the heart with the potential to both directly and indirectly modulate cardiac electrophysiology. Patch clamp analysis of fibroblasts have identified a wide variety of membrane ionic conductances. However, potential regional differences within this population of cells with regard to heart chamber have not been emphasized. More importantly, there is limited information concerning how fibroblast membrane currents are altered with cardiac disease. In addition, although it remains controversial whether myocytes and fibroblasts electrically couple in the intact heart, there is strong *in vitro* evidence of electrical interactions between these two cell types. Finally, recent studies from our laboratory have demonstrated the potential of fibroblasts to

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modulate cardiac electrophysiology may be enhanced with cardiac injury. Continued work in this area over the next several years from our laboratory and others may provide important mechanistic information on the role of fibroblasts in the development of arrhythmias associated with cardiac disease. Moreover, these studies may identify particular aspects of the fibroblast activation process as new targets for antiarrhythmic therapy.

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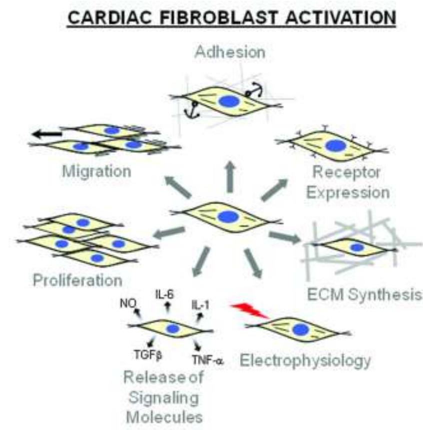

Figure 1. Functional consequences of fibroblast activation

Fibroblast transformation to the myofibroblast phenotype is associated with important changes in the biological behavior of these cells. Limited information is available regarding the electrophysiological consequences of this transition.

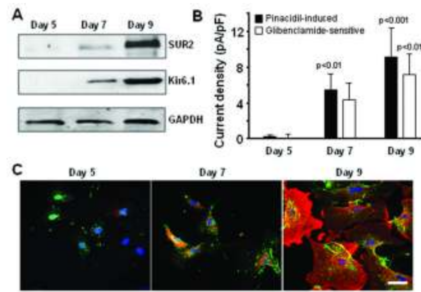

Figure 2. K_{ATP} channel activity in cardiac fibroblasts

A, SUR2 and Kir6.1 protein expression in fibroblast cultures. Western blots were performed with whole cell lysates of cultured mouse ventricular fibroblasts at 5, 7 and 9 days after isolation. GAPDH was used as a loading control. **B**, Pinacidil-induced and glibenclamide-sensitive average current density in fibroblasts evaluated at 5, 7 and 9 days of culture. Current amplitude was measured as the average value obtained during a 10 s pulse at 50 mV from a holding potential of −50 mV in the whole cell configuration. Current amplitude was normalized to cell capacitance. Probability values correspond to comparisons to 5 days in culture. **C**, Fibronectin (green) and α-SMA (red) expression in cultured fibroblasts at different times in culture. Bar is 50 µm. (Modified with permission from Benamer N, Moha Ou Maati H, Demolombe S, Cantereau A, Delwail A, Bois P, Bescond J, Faivre JF. Molecular and functional characterization of a new potassium conductance in mouse ventricular fibroblasts. J Mol Cell Cardiol. 2009;46(4):508–517.)

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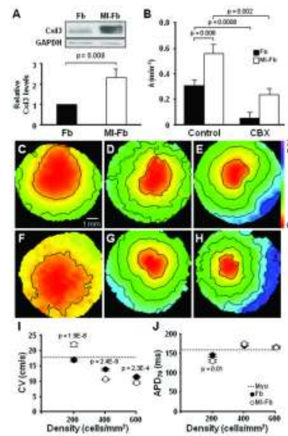

Figure 3. Cardiac injury enhances fibroblast-myocyte interactions

A, Representative immunoblot and quantification of relative Cx43 levels in cultured fibroblasts isolated from normal (Fb) and infarcted (MI-Fb) hearts. Protein expression levels were normalized to Fb. GAPDH was used as a loading control. **B**, Average permeability constants (*k*) obtained with gap-FRAP from myocytes plated on top of Fb and MI-Fb monolayers under control conditions and in the presence of 200 μM carbenoxolone (CBX). **C–E**, Representative activation maps from heterocellular cultures of myocytes and Fb plated on top. Fb were plated at 200, 400, and 600 cells/mm^2, respectively. Lines are 10 ms isochrones. **F–H**, Representative activation maps from heterocellular cultures of myocytes and MI-Fb plated at the same densities as panels C–E. **I**, Average CV of the heterocellular cultures for different fibroblast plating densities. Dotted line corresponds to average CV of homocellular myocyte monolayers (Myo). Closed and open symbols correspond to heterocellular cultures with Fb and MI-Fb, respectively. Probability values correspond to significant differences between Fb and MI-Fb at the same density. **J**, Average APD_{70} of heterocellular cultures for different fibroblast plating densities. Dotted line corresponds to average APD_{70} of Myo. Probability value corresponds to significant difference between Fb and MI-Fb at the same density. (Modified with permission from Vasquez C, Mohandas P, Louie KL, Benamer N, Bapat AC, Morley GE. Enhanced Fibroblast-Myocyte Interactions in Response to Cardiac Injury. Circ Res. 2010; 107(8):1011–1020.)

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Table 1

Membrane Currents Found in Adult Cardiac Fibroblasts.

Physiological Condition	Recording Condition	Atria	Ventricle
Normal Tissue	Acutely Isolated	• Mechanosensitive non-selective cation current38	• Inward rectifier K^+ current41 • Voltage dependent outward K^+ current41, 42 • Non-selective cation current43
	Cultured	• Not studied	• Non-selective cation current44 • $Ca2^+$ activated K^+ current45, 46 • Delayed rectifier K^+ current46 • Outward K^+ current41, 46 • Inward rectifier K^+ current41, 46 • Cl^- current46 • Voltage-gated Na^+ current46 • ATP-sensitive K^+ current34
Diseased Tissue	Acutely Isolated	• Non-selective cation current48	• Not studied
	Cultured	• Voltage activated proton current47	• Not studied