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Myocyte-Fibroblast Communication in Cardiac Fibrosis and Arrhythmias: Mechanisms and Model Systems

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Abstract

Development of cardiac fibrosis and arrhythmias is controlled by the activity of and communication between cardiomyocytes and fibroblasts in the heart. Myocyte-fibroblast interactions occur via both direct and indirect means including paracrine mediators, extracellular matrix interactions, electrical modulators, mechanical junctions, and membrane nanotubes. In the diseased heart, cardiomyocyte and fibroblast ratios and activity, and thus myocyte-fibroblast interactions, change and are thought to contribute to the course of disease including development of fibrosis and arrhythmogenic activity. Fibroblasts have a developing role in modulating cardiomyocyte electrical and hypertrophic activity, however gaps in knowledge regarding these interactions still exist. Research in this field has necessitated the development of unique approaches to isolate and control myocyte-fibroblast interactions. Numerous methods for 2D and 3D co-culture systems have been developed, while a growing part of this field is in the use of better tools for *in vivo* systems including cardiomyocyte and fibroblast specific Cre mouse lines for cell type specific genetic ablation. This review will focus on (i) mechanisms of myocyte-fibroblast communication and their effects on disease features such as cardiac fibrosis and arrhythmias as well as (ii) methods being used and currently developed in this field.

Keywords

Cardiomyocyte; Fibroblast; Fibrosis; Arrhythmia; Cell-cell interactions

1.1. Introduction

Cardiomyocytes and fibroblasts are key cell types in the heart that communicate to regulate normal cardiac function as well as the heart's response to pathogenic stimuli [1, 2]. They can communicate indirectly and directly to regulate cardiac cell signaling and responses via

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None.

paracrine factors, extracellular matrix (ECM) interactions, electrical modulators, and mechanical interactions [1, 2]. Loss of myocyte-fibroblast communication is thought to play a central role in heart disease, especially during end-stage manifestations, which include cardiac fibrosis and arrhythmias. The increased deposition of ECM and fibroblast accumulation in cardiac fibrosis alters myocyte-fibroblast homeostasis as both ECM to myocyte and fibroblast to myocyte ratios are increased [3, 4]. Arrhythmias specifically arise from decoupling of cardiomyocytes, which then leads to conduction slowing, irregular conduction propagation, defects in source-sink activity, and conduction block [5–7]. However, recent *in vitro* evidence highlights the presence of molecular machinery electrically coupling myocytes to fibroblasts as well as importance of fibroblasts on electrical propagation and conduction of cardiomyocytes, suggesting a potential role in arrhythmias [8]. Thus, a better understanding of myocyte-fibroblast communication is critical in both atrial and ventricular arrhythmias since atrial fibrillation and ventricular tachycardia are the most common causes of cardiac morbidity and sudden cardiac death, respectively [4, 9–11]. Gaps also exist in identifying ideal model systems to decouple or isolate primary defects of how myocyte-fibroblast communication can go awry in these disease processes. Much of the early research in the field has focused on dissecting the role of individual cell types, i.e. either cardiomyocytes in isolation or fibroblasts in isolation [12, 13]. Newer research has begun to isolate and study the importance of myocyte-fibroblast interactions through a variety of *in vitro* 2D and 3D co-culture systems as well as *in silico* computational models [12, 14, 15]. A growing field has been the use of genetic mouse models, where cardiomyocyte-specific and fibroblast-specific Cre models are being exploited and developed to better understand the effect of gene ablation in a cardiomyocyte and fibroblast cell-type specific manner *in vivo* [16, 17]. This review will focus on the mechanisms regulating myocyte-fibroblast communication and their impact on cardiac fibrosis and arrhythmias, with a particular emphasis on current methods used in this field.

1.2. Fibroblast Functions In the Heart

The mammalian heart is composed of a number of cell types including cardiomyocytes, fibroblasts, endothelial cells and vascular smooth muscle cells [1, 2, 18]. Though cardiomyocytes make up the majority of the heart by volume, fibroblasts are relatively higher in in numbers when compared to cardiomyocytes [19, 20]. Fibroblasts are of mesenchymal origin [1] and play an essential role in ECM production and remodeling as well as have additional roles in modulating the myocardial response to changes in electrical and chemical signaling [1]. Specifically, cardiac fibroblasts produce and degrade ECM components, which include collagens, proteoglycans, glycoproteins, cytokines, growth factors, and proteases. The ECM acts as the structural network and signaling mediator in the heart and as such, modulation of ECM properties can lead to drastic changes in cardiac function, which has been reviewed in this issue [21] and elsewhere in detail [22–24].

In the diseased state, cardiac fibroblasts become activated and differentiate to myofibroblasts [3, 25–29]. Myofibroblasts play a prominent role in the heart after injury. Their cell characteristics are distinct from conventional cardiac fibroblasts, which include expressing high levels of exocytic vesicles, smooth muscle actin-positive stress fibers as well as specialized adhesion complexes resulting in a contractile phenotype [30, 31]. As a result,

myofibroblasts secrete high levels of pro-inflammatory and pro-fibrotic paracrine factors as well as ECM proteins. This is observed in the heart as a disproportionate increase in ECM amount and changes in ECM quality, which altogether promote fibroblast proliferation ensuing in cardiac fibrosis [32, 33].

Major factors contributing to cardiac fibrosis include mechanical pressure overload, cardiac injury, genetic predisposition due to congenital heart disease and age [34, 35]. Thus in cardiac disease and injury states, cardiac fibroblasts display altered expression of ECM components as well as matrix metalloproteinases (MMP), which are the enzymes that modify and degrade ECM. Activation of ECM and MMP production in cardiac fibroblasts is mediated by various signaling pathways, with two of the most well characterized being transforming growth factor β1 (TGF-β1) and angiotensin II (AngII) signaling pathways [36–38]. Other signaling pathways that contribute to ECM and MMP production in cardiac fibroblasts include endothelin-1, fibroblast growth factor 2, connective tissue growth factor, platelet-derived growth factor, insulin-like growth factor, as well as various interleukins, which have been reviewed in detail elsewhere [1, 35, 39]. Cardiac fibrosis greatly increases the propensity of the myocardium to become arrhythmic, further highlighting the contribution of derailed myocyte-fibroblast communication in cardiac arrhythmias [5].

1.3. Mechanisms of cardiomyocyte-fibroblast communication

Cardiomyocytes and fibroblasts can communicate indirectly and directly via (i) paracrine mediators, (ii) ECM interactions, (iii) electrical modulators, (iv) mechanical junctions, and (v) membrane nanotubes with downstream effects on cardiac hypertrophy, fibrosis and arrhythmias (Figure 1).

1.3.1. Paracrine Mediators

Paracrine mediators allow for cardiomyocytes and fibroblasts to indirectly communicate as these factors can be secreted from one cell type and diffuse and circulate onto the other cell type. The majority of the focus in this field has been on the effects on TGF-β1 and angiotensin II signaling pathways affecting myocyte-fibroblast communication in the context of cardiac fibrosis, hypertrophy and arrhythmias. More recent work has identified additional paracrine factors that involve interleukins and the Wnt signaling pathway as it may affect myocyte-fibroblast communication in the context of cardiac hypertrophy and fibrosis. In this section we will discuss the role of TGF-β1, angiotensin II, interleukins, and Wnt signaling in relation to myocyte-fibroblast communication.

A major paracrine factor regulating myocyte-fibroblast communication in the setting of cardiac fibrosis is TGF-β1. TGF-β1 and its receptors (TGF-β receptor 1 (TGFβR1) and TGFβR2)) are expressed in cardiomyocytes and fibroblasts [40–43]. In fibroblasts, TGF-β1 treatment increases fibroblast differentiation into myofibroblasts and increases their ECM production [44, 45], recapitulating the fibrotic pathway. *In vivo* studies that downregulate TGF-β1 have also demonstrated that suppression of TGF-β signaling is associated with loss of fibrosis, which highlights preferential effects of TGF-β signaling on cardiac fibroblasts *in vivo* [46, 47]. Interestingly, Koitabishi *et al* showed that cardiomyocyte-specific loss of TGFβR2 (and thus loss of TGF-β signaling) in mice *in vivo* also resulted in reduced fibrosis

in the setting of pressure overload, highlighting that loss of myocyte-derived TGF-β signaling was sufficient to blunt the function of cardiac fibroblasts *in vivo* [46]. Studies performed in a mouse model overexpressing TGF-β1 in atrial and ventricular cardiomyocytes revealed increased fibrosis and arrhythmias in the atria but not ventricles of the heart [48], suggesting that there may be added underlying complexities with how myocyte-fibroblast communication may be regulated in atrial versus ventricular muscle as well as their impact on fibrotic and arrhythmogenic pathways.

Angiotensin II (AngII) is also thought to be a major paracrine factor promoting myocyte-fibroblast communication, and whose functions can intersect with TGF-β1 signaling pathways. Specifically, *in vitro* co-culture studies and cardiomyocyte monolayer studies with fibroblast-conditioned media demonstrated that fibroblasts were required for the Ang-II mediated cardiomyocyte hypertrophic response [14, 49–51]. Studies performed in AngII-treated TGFβ-1 deficient mice further demonstrated that TGFβ–1 is required for the AngII-mediated hypertrophic response *in vivo* [52]. It was hypothesized that AngII may mediate preferential effects on fibroblasts because expression of angiotensin type I receptor is higher in fibroblasts versus cardiomyocytes [50]. These effects could translate to arrhythmias as AngII has been shown to also have a number of direct effects on cardiomyocyte electrophysiology, as reviewed elsewhere [53]. Future studies focused on better understanding the role of Ang II in pro-arrhythmic settings will be required to determine if these effects could be mediated by myocyte-fibroblast interactions.

Interleukins may also act as signaling mediators between cardiomyocytes and fibroblasts as they are expressed by and affect both cell types. Leukemia inhibitory factor (LIF) and cardiotrophin-1 (CT-1), which are cytokines from the interleukin (IL)-6 family, are both expressed in cardiomyocytes and fibroblasts [54]. In cardiomyocytes, both LIF and CT-1 can stimulate cardiomyocyte hypertrophy [55–57]. In fibroblasts, LIF and CT-1 treatment can increase fibroblast proliferation [55–57]. Interestingly, LIF treatment can also inhibit fibrotic activation by blocking differentiation of fibroblasts to myofibroblasts as well as reducing collagen secretion [56]. Interleukin 33 (IL-33), which is a member of the IL-1 family, is also produced by fibroblasts [58, 59]. In cardiomyocytes, IL-33 can inhibit AngII and phenylephrine induced cardiomyocyte hypertrophy *in vitro* [59]. *In vivo* studies demonstrated that IL-33 administration inhibited cardiac hypertrophy as well as reduced fibrosis after pressure overload [59], suggesting that IL-33 may function as a paracrine signal produced by fibroblasts to modulate cardiomyocyte responses to hypertrophic stimuli.

Wnt signaling has also been implicated in mediating myocyte-fibroblast communication. Wnt1 is upregulated in cardiac fibroblasts after ischemia-reperfusion injury [60]. Global inhibition of Wnt signaling (thus in both cardiomyocytes and fibroblasts) in mice *in vivo* resulted in reduced fibrosis and improved cardiomyocyte/cardiac recovery after myocardial infarction [61–64]. Interestingly, cardiomyocyte inhibition of Wnt signaling via overexpression of an endogenous inhibitor of Wnt, Sfrp1, *in vivo* resulted in an opposite response, which included increased fibrosis and worse cardiomyocyte/cardiac recovery after myocardial infarction [65]. These results suggest that Wnt signaling in fibroblasts may directly regulate cardiomyocyte survival and thus, myocyte-fibroblast crosstalk may underlie

the distinct responses observed in the heart in response to global versus cardiomyocyte specific inhibition of Wnt signaling in the setting of cardiac injury.

1.3.2. ECM interactions

The well established role of ECM is to provide structural support for the cells of the heart. More recently, it has become evident that ECM proteins can also function as signaling mediators between cardiomyocytes and fibroblasts. Fibroblasts are the primary cell types that produce and secrete ECM components (eg., collagen and fibronectin) as well as the enzymes that degrade ECM components (eg., MMPs and tissue inhibitors of MMPs (TIMPs)) [66, 67]. These proteins can in turn be sensed by cells in close proximity, such as cardiomyocytes [66, 67].

Fibroblasts produce and secrete collagens, which can then directly interact with cardiomyocytes [68, 69]. Alterations in collagen levels and secretion from fibroblasts have been directly associated with alterations in cardiomyocyte function. Specifically, increased levels of collagen in the heart in the form of excess ECM production due to cardiac fibrosis can lead to impaired cardiomyocyte connectivity and function [70]. In addition, alterations in the ratio of the predominant collagen subtypes (collagen I and III) in the heart can also impact cardiomyocyte function, based on their differing physical properties. Diseased hearts exhibiting dilated cardiomyopathy (DCM) and ensuing diastolic dysfunction, display alterations in collagen composition (increased collagen type I to collagen type III ratio), which effects cardiomyocyte compliance [71, 72].

Fibronectin is another major ECM component that is produced by fibroblasts and that can be sensed by cardiomyocytes. Cardiomyocytes specifically interact with fibronectin on the cell surface via integrins which has been reviewed in this issue [73] and elsewhere [74, 75]. Though there are no studies that directly assess the functional role of fibronectin in the heart, findings from mouse models targeting members of the integrin complex suggest that cardiomyocyte disruption of fibronectin related interactions have detrimental effects on cardiac electrophysiology and function [74]. Specifically, cardiomyocyte-specific loss of vinculin, the protein that links the actin cytoskeletal network to integrins, can lead to cardiac arrhythmias and sudden death in the absence of heart failure [76]. These findings suggest that integrin signaling alterations may be sufficient to generate a substrate for cardiac arrhythmias; however, the importance of the integrin-fibronectin interaction in this pathway remains to be examined.

Fibroblasts and cardiomyocytes both produce MMPs, which are the enzymes that degrade extracellular ECM components and TIMPs that inhibit the actions of MMPs [77]. Evidence from co-culture systems suggests that MMPs are critical in improving fibroblast driven effects on cardiomyocyte organization. In this system, it was found that cardiomyocytes cultured with fibroblasts exhibited improved alignment compared to cardiomyocytes cultured alone and that this improvement was abrogated by MMP inhibition [78]. This interaction may also have relevance to the diseased heart as evidence from *in vivo* models of DCM and post-MI remodeling reveal that MMP activity is elevated, and that inhibition of MMPs can improve recovery of the heart [79–81]. Though more work remains to be done to demonstrate the impact of a direct interaction, these findings suggest that there may be an

important contribution of MMP dependent cross-talk between fibroblasts and cardiomyocytes in the diseased heart.

1.3.3. Electrical Modulators

Cardiomyocytes and fibroblasts are also able to directly interact via gap junctions. At the single channel level, whole patch clamp studied homotypic and heterotypic gap junctions in cell pairs from co-cultured neonatal rat cardiomyocytes and fibroblasts identified that gap junctions functionally form between these cell types and display properties of hemichannels, in that they have proportionately lower conductance suggesting a channel formed from two different types of connexins reflective of the two different cell types [82]. However, findings from other groups that have exploited 2D and 3D co-cultures have identified connexin 43 plaques and expression between ventricular cardiomyocytes and fibroblasts [83, 84]. These findings are contiguous with cardiomyocytes from the working myocardium, which dominantly express connexin 43 at gap junctions [83]. Interestingly, studies performed in rabbit sinoatrial node cardiomyocytes and fibroblasts revealed that connexin 40 was predominantly expressed between fibroblasts; however, when sinoatrial node fibroblasts were in close proximity and coupled to myocytes they now predominantly expressed connexin 45, highlighting effects of myocyte-fibroblast coupling on gap junction expression and function [83, 85].

An additional type of cell-cell connection that may have effects on electrical connectivity between cells are membrane nanotubes, or tunneling nanotubes, which were first observed in cultured PC12 cells [86]. Membrane nanotubes are long thin membrane-bound connections that carry membrane components, Ca^{2+}, mitochondria, and other cargo between cells [87]. These structures have been identified between cardiomyocytes and fibroblasts both *in vitro* and *in vivo* [88]. Though research remains to be done to establish the full function of membrane nanotubes, their presence between cardiomyocytes and fibroblasts and established ability to carry ions in other systems is supportive of a role in myocyte-fibroblast interactions.

From an electrophysiological standpoint, these electrical connections can have a significant role in cardiac conduction. Fibroblasts themselves are unable to generate action potentials, however they can exhibit conductive properties. In culture, fibroblasts can exhibit rhythmic depolarization along with cardiomyocytes and affect electrical synchrony as well as alter automaticity parameters [89–92]. Fibroblasts can even display stretch dependent changes in conductance and cation flux [90, 93, 94]. Theoretical models have described the electrical interactions between cardiomyocytes and fibroblasts in terms of zero, single-sided and double-sided degree of coupling and have also proven useful in computational systems. Zero-sided coupling, occurs when fibroblasts are functionally insulated and do not couple to cardiomyocytes via gap junctions. Single-sided coupling occurs when fibroblasts connect to an electrically interconnected group of cardiomyocytes via gap junctions. In this type of coupling, fibroblasts have a strong buffering effect (electrotonic load). Double-sided coupling occurs when fibroblasts connect to myocytes such that not all myocytes are in direct contact with each other, thus acting as the bridge to allow fibroblasts to form the conducting pathways. Heart tissue may display a combination of these interactions and

alterations in fibroblast density or activity may shift the coupling mechanism that dominates [95, 96].

1.3.4. Mechanical Junctions

Mechanical junctions are found at distal ends of the cell along with gap junctions, which altogether encompass the intercalated disc in cardiomyocytes [97]. These structures include fascia adherens and desmosomal cell-cell junctions, which play an important role in structural support of cardiac muscle, especially during stress, as well as lateral force transmission during cardiac contraction. Interestingly, components from both the adherens junction and desmosome, such as N-cadherin and desmoplakin, respectively, have been identified at sites of myocyte-fibroblast interactions in co-culture studies, suggesting the possibility of direct mechanical interaction between both cell types [84, 98]. Furthermore, cardiomyocyte restricted genetic mouse models where mechanical junctions are disrupted also display increased cardiac fibrosis and arrhythmias [17, 99, 100], suggesting the possibility that loss of these interactions may play a contributory role in cardiac disease. The role of mechanical junctions in myocyte-fibroblast interactions may also be important to assess in human cells as mutations in desmosomal genes are associated with the human sudden cardiac death syndrome, arrhythmogenic right ventricular cardiomyopathy [97, 101].

Myofibroblasts and cardiomyocytes may interact through direct mechanical forces. Mechanical junctions have been observed both between myofibroblasts as well as between myofibroblasts and cardiomyocytes [102, 103]. In a myofibroblast-cardiomyocyte co-culture setting, conduction velocity is reduced but can be increased using contraction decouplers and mechanosensitive ion channel blockers [102]. Also in co-culture settings, TGF-β1 stimulated myofibroblasts are able to exert tonic contractile forces on neighboring cardiomyocytes leading to increased mechanosensitive channel openings and slowing of conduction [102]. These results suggest the presence of mechanoelectric interactions, which may occur via mechanical stress activation of fibroblast ion channels [102, 104], mechanical stress activation of cardiomyocyte channels [102, 105], or mechanical stress induced release of paracrine factors [106]. Along with gap junction connections between fibroblasts and cardiomyocytes, mechanical forces could communicate signals between the two cell types directly.

1.4. Model systems used to dissect cardiomyocyte-fibroblast interactions

In vitro, *in vivo* and *in silico* models systems have been used to dissect myocyte-fibroblast communication. These include two dimensional (2D) and three dimensional (3D) co-culture systems, genetic mouse models and computational models. In the following section, we will describe advantages, limitations and applications of these models (Table 1).

1.4.1. *In Vitro* Model Systems

Myocyte-fibroblast interactions have been conventionally assessed using 2D co-culture model systems as they present as simple and cost-effective. Briefly, cardiomyocyte and fibroblast mixtures are seeded onto a cell culture dish, resulting in a random and non-patterned distribution of myocyte-fibroblast interactions. The impact of direct and indirect

(paracrine) interactions between myocytes and fibroblasts can be measured *in vitro* through various readouts. Readouts include measuring (i) secretion of proteins/paracrine effects in the environment of myocyte-fibroblast interactions, (ii) expression of various proteins at sites of myocyte-fibroblast interactions and (iii) electrophysiological properties (action potential, field potential, beating rate, electrical coupling) of myocyte-fibroblast interactions (direct and indirect) [14, 107, 108]. 2D co-culture studies were pivotal in identifying the paracrine effects of TGF-β on AngII-mediated functions in cardiac fibroblasts. Specifically, it was shown that myocyte-derived factors increased Ang II-induced collagen expression in cardiac fibroblasts [14, 107]. Paracrine effects can also be dissected in transwell cultures where cardiac fibroblasts and myocytes are physically separated; however, remain in indirect contact via the media [109]. Newer technologies (electric cell-substrate impedance sensing system) are also being applied onto conventional 2D co-culture models to more rigorously assess the contractile and electrophysiological properties of myocyte-fibroblast interactions in a syncytium and in real time [110]. For example, fibroblast to myocyte ratios can be manipulated in a culture dish setting to assess their role on global cardiomyocyte contractility and communication [110]. Although much of current knowledge on fibroblasts has been obtained from the 2D culture system, it should be noted that fibroblasts undergo rapid transformation to myofibroblasts when grown on rigid substrates [111, 112]. It is therefore likely that most of our understanding on the function of “fibroblasts” has been obtained using myofibroblasts due to lack of proper identification of cell type in these studies. However, based on the elevated presence of myofibroblasts in the diseased heart, these studies employing the 2D culture system may shed light on important pathways in disease pathogenesis [3, 25–29].

Patterned 2D co-cultures using microfabrication technologies are now being exploited to spatially separate and characterize the molecular and physiological attributes of myocyte-fibroblast interactions. This is advantageous as it provides for spatial control of cell-cell interactions. Microfabrication is the process of fabricating miniature structures at the micrometre and nanometer scale. Microfabricated silicon combs provide an avenue to fully or partially separate cell types from one another [113]. Silicon combs have been used to study skeletal myocyte-fibroblast interactions [114]. An example of its use included studying the effects of myocyte-fibroblast interactions on myotube differentiation and alignment [114]. Photolithography can be used to spatially separate cell types by exploiting the differential adhesive properties of different cell types [115, 116]. Previous techniques exploited photolithography and microfluidics to structurally pattern collagen onto cell culture surfaces to create cardiomyocyte and fibroblast adhesive regions [115]. These studies demonstrated that these structured myocyte-fibroblast co-cultures recapitulated *in vivo* ventricular tissue organization [115]. More recent studies have exploited photolithography to pattern different extracellular matrices onto cell culture surfaces [116]. Briefly, i) a fibroblast adhesive agar-coated glass coverslip is coated with a photoresistor compound, ii) the compound is selectively removed to reveal the underlying agar layer, iii) a cardiomyocyte adhesive surface is layered on the exposed agar, iv) the remaining photoresistor compound is removed leaving a pattern of cardiomyocyte adhesive regions and fibroblast adhesive regions, and v) cardiomyocytes are sequentially plated to allow for their attachment prior to fibroblasts, which are plated 24 hours later [116]. This technique has been exploited to better

understand electrical coupling between myocytes and fibroblasts [117]. Other usage of micropatterning cell surfaces include polymer-based microfluidics and soft lithograghy, which have been reviewed in detail elsewhere [12, 118, 119], but remain to be exploited to better understand myocyte-fibroblast interactions.

3D co-cultures provide an environment that is thought to mimic the complexity of tissue since intercellular networks and interactions are promoted in 3D [12]. Scaffold-based and scaffold-free 3D co-culture systems are being exploited to study myocyte-fibroblast interactions in an artificial or native ECM environment, respectively. Scaffold-based methods employ biomaterials such as porous and fibrous hydrogels [120, 121] as well as nanofibers [122]. These biomaterials create a structure to attract cells to interact within a 3D matrix in order to study the impact of cell-cell and cell-ECM biology in a tissue like setting. For example, GelMA hydrogels have been used as a scaffold to study the effect of myocyte-fibroblast (connexin 43) and fibroblast-ECM (β1-integrin) interactions on synchronous contraction [123]. In contrast to scaffold-based systems, in which cell-ECM interactions dominate [123], cell-cell interactions are maximized in self-assembling scaffold-free 3D cultures. For this technique, cardiomyocyte and fibroblast mixtures are seeded onto low-attachment surfaces or other special surfaces to promote cell-cell interactions, which generate spherical cardiac micro-tissues [124]. Myocytes and fibroblasts were found to self-assemble and intersperse in a tissue-like distribution as well as express cardiac ECM and contractile proteins (e.g. Ca2+ handling proteins). In addition, the micro-tissues were found to be functionally competent as they exhibited spontaneous action potentials and contractions [124]. Modifications in attachment surfaces have provided key improvements in controlling spherical size, resulting in the ability to assess larger and more complex micro-tissues [124].

Much of the current research on better understanding myocyte-fibroblast interactions has focused on exploiting mouse and rat cardiomocytes and fibroblasts in 2D and 3D co-culture settings. However, myocyte-fibroblast interactions also play a key role in humans. Evidence from human embryonic stem cell derived cardiomyocytes highlights that fibroblast interactions (via co-culture or conditioned media) are key drivers of human cardiomyocyte differentiation [125–128]. These interactions may also contribute to human cardiac disease settings, where defects in myocyte-fibroblast coupling may underlie cardiac fibrosis and arrhythmias, which are highly prevalent. Given the increasing number of hiPSC-based models of cardiac disease [129] and the advent of 3D engineered tissue models using hiPSCs [130], future studies focused on better understanding the impact of myocyte-fibroblast communication in these 2D and 3D model systems would be of significant interest to better understand their contribution to human disease settings.

1.4.2. *In Vivo* Model Systems

Slices of living cardiac tissue from humans and animal models are also being proposed as an experimental model system to better understand cell-cell communication in the context of the diseased heart [131–133]. This experimental system presents some advantages in that it is (i) directly relevant to humans, (ii) provides an environment that retains the native cytoarchitecture of myocyte-fibroblast interactions in the context of disease and (iii) can be

exploited for pharmacological drug screening. However, several limitations exclude its prominent use to dissect the role of myocyte-fibroblast interactions in the context of disease as (i) there are lack of healthy controls for comparative studies, (ii) invasive procedures are required to obtain these tissues and (iii) the contribution of individual cell types in the context of these interactions are difficult to isolate and assess.

Conditional genetic mouse models that exploit cardiac and fibroblast cell specific gene targeting approaches can be used in isolation or combination with culture studies as a powerful *in vivo* model system to help dissect the impact of myocyte-fibroblast interactions in cardiac disease conditions *in vivo*. Periostin-Cre, has been exploited for its fibroblast-specific expression pattern in the heart following pressure overload [16]. Fibroblast-specific deletion of *Klf5*, which is a member of the Krüppel-like factor family, in mice *in vivo* using the periostin-Cre resulted in a blunted response to pressure-overload induced cardiac hypertrophy as well as fibrosis [16]. On the other hand, cardiomyocyte-specific deletion of *Klf5* in mice *in vivo* using alpha-myosin heavy chain Cre, resulted in a normal hypertrophic and fibrotic response to pressure overload similar to controls [16]. These results suggested that fibroblasts can directly communicate with myocytes to regulate hypertrophic responses [16]. However, periostin is thought be expressed at low levels in the adult heart [134], thus its use may be restricted to fibroblasts in stress states such as pressure overload [16]. Kong *et al* recently explored the expression pattern of the fibroblast-specific protein 1 (FSP1)-Cre mouse line using a green fluorescent protein reporter in the context of a fibrosis model and showed low specificity for fibroblasts as more than 30% of FSP1 positive cells were hematopoietic cells, endothelial cells, or vascular smooth muscle cells [134]. As a result, these studies demonstrate that none of the currently known fibroblast promoters are active in all fibroblasts in the heart. Thus, future studies focused on identifying a universal biochemical marker for fibroblasts are needed to propel research in this area forward.

1.4.3. *In Silico* Model Systems

Ionic, cellular (2D) and whole heart (3D)-based computational models can be used to simulate structural and electrical properties of cardiomyocytes and fibroblasts in the setting of cardiac fibrosis and arrhythmias.

Early studies exploited ionic and cellular based model systems to better understand the interactions between myocytes and fibroblasts in the setting of arrhythmias. In these models, cardiomyocyte and fibroblast membrane electrical properties such as ion channels, exchangers, and pumps as well as cellular signaling can be simulated [135–139]. These simulations have been extended to studying electrical properties of human cardiomyocytes [140]. In terms of fibroblasts, two models are used for simulations, which include passive and active properties of fibroblasts [141]. In passive model, membrane capacitance is connected in parallel to ohmic resistance, which can be used to manipulate fibroblast resting membrane potential and membrane conductance parameters [142, 143]. The first passive fibroblast model was used to simulate sino-atrial myocytes as cell pairs by Noble’s group [142]. Extension of computational models to two-dimensional sheets was pioneered by Winslow and colleagues [136]. In the active model, properties of four membrane currents, which include the potassium current (inward rectifying and time and voltage dependent

rectifying), electrogenic sodium-potassium ATPase and sodium conductance were used to generate simulations [143]. Passive and active fibroblast models can then be combined with cardiomyocyte models to better understand myocyte-fibroblast coupling in an environment free from secondary disease manifestations [143]. Specifically in the passive model, a small change in action potential duration was observed in cardiomyocytes when coupled to fibroblasts. However, in the active model, a striking decrease in action potential duration was observed in cardiomyocytes when coupled to fibroblasts and fibroblasts showed an electrotonic depolarization which may indicate alterations in contraction [143]. Brown et al have used these myocyte-fibroblast models to better understand the effects of coupling on many other electrical properties of these cells in pro-arrhythmic settings [141].

Recent studies have exploited computational models to multi-scale levels in order to explore the contribution of myofibroblast-myocyte coupling in atrial and ventricular arrhythmias in vivo [144]. Three-dimensional models of the heart are being reconstructed from high-resolution magnetic resonance (MR) imaging of live animals and humans [145, 146]. These 3D models preserve the geometry and fiber orientations observed in the native atria and ventricle of the heart [144, 146]. Myocyte-fibroblast coupling models at the cellular scale are then introduced into these 3D models in order to characterize how myocyte-fibroblast interactions impact the electrophysiological properties of the heart *in vivo* [144]. For example, these models could simulate the reduction of ion currents (I_{Na}, $I_{Ca,L}$, I_{Kr} and I_{Ks}) and the impact of different ratios of infiltrating myofibroblasts on the peri-infarct zone as was observed in a rabbit model of myocardial infarction. They found that the propensity for arrhythmias was bi-directional and changed at intermediate and high densities of infiltrating myofibroblasts [144]. These results improved our knowledge of the role of myofibroblasts in arrhythmia generation.

However some controversy exists in the field as different fibroblast-myocyte interactions are being used for simulations and these different models impact the response on conduction [147, 148]. Parallel studies performed in *in vitro* and *in vivo* biological systems may help validate the nature of myocyte-fibroblast interactions and conclusions from these simulations. Thus, more refined and standardized models should be considered to more accurately depict myocyte-fibroblast interactions. Models that portray these interactions in the human heart should also be considered.

1.5. Conclusions and Future Directions

Identifying the impact of fibroblasts on cardiomyocyte actions has been a growing research area given that there is increasing knowledge of their communication via biomechanical (mechanical junctions, extracellular matrix), electrical (gap junctions and membrane nanotubes) and biochemical (paracrine factors) means. Numerous studies suggest that crosstalk between these cell types plays an important contributory role in cardiac fibrosis and arrhythmias. However, many gaps remain in this field and methods are constantly evolving to better understand the primary actions and consequences of these interactions. Floxed mouse models could be better exploited in the future to improve current *in vitro* systems as they could allow for isolation of cell-type specific roles of these communicating signals. For example, selective ablation of genes in either cardiomyocytes or fibroblasts in

co-culture settings could be performed using viral-mediated Cre strategies in floxed cardiomyocytes co-cultured with wild type fibroblasts versus floxed fibroblasts co-cultured with wild type cardiomyocytes. This could also be applied to *in vivo* settings, where there is a growing need for better fibroblast-specific Cre models to isolate and better understand the impact of targeting gene ablation in fibroblasts on cardiomyocyte function. Currently there is also a lack of human model systems to better understand myocyte-fibroblast interactions. Use of human induced pluripotent stem cell-derived cardiomyocytes and fibroblasts can be exploited in the future to confirm mechanisms in humans, especially in disease settings as well as electrophysiological parameters, which are known to be significantly different in some respects from mice.

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Highlights

- Myocyte-fibroblast interactions are important in normal heart function as well as in development of disease phenotypes such as cardiac fibrosis and arrhythmias.
- These interactions take place through various modalities including paracrine mediators, extracellular matrix interactions, electrical modulators, and mechanical junctions.
- Numerous unique approaches have been developed to isolate and control myocyte-fibroblast interactions in systems ranging from computational models to co-culture systems to *in vivo* and *ex vivo* animal systems.

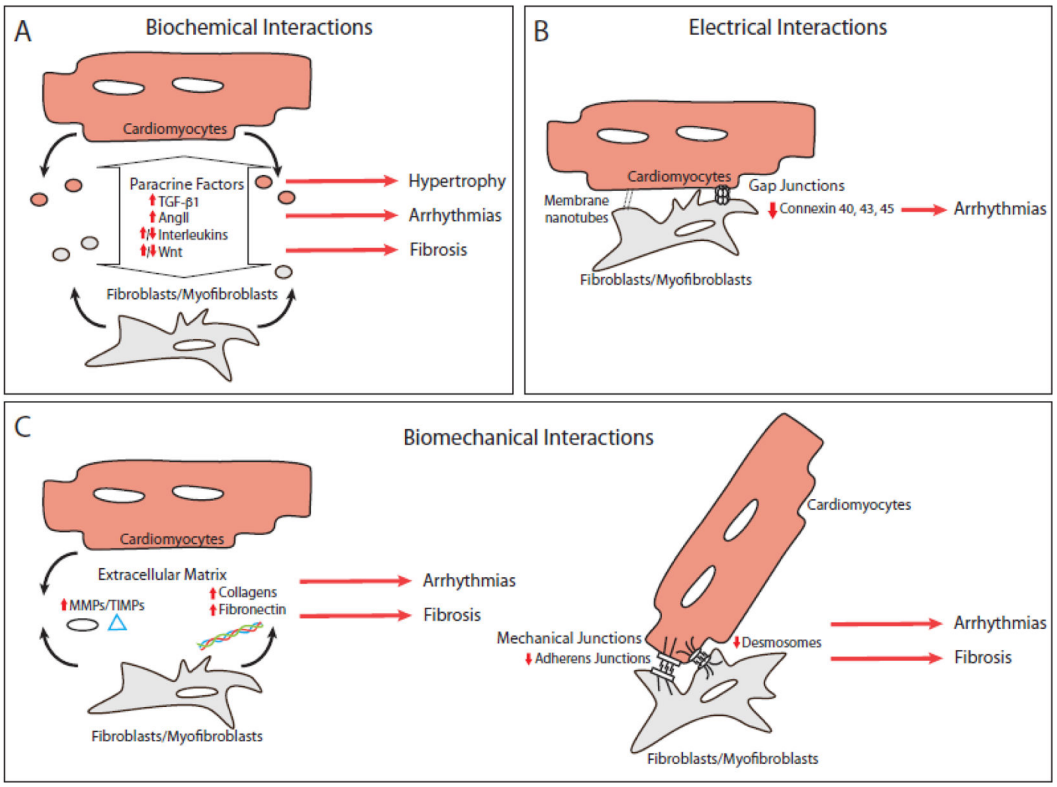

Figure 1.

Cardiomyocyte-fibroblast interactions. Various interactions between cardiomyocytes and fibroblasts in the heart are diagrammed above with emphasis on the following categories: (A) biochemical (paracrine signals), (B) electrical (connexins and membrane nanotubes), and (C) biomechanical (ECM and mechanical junctions). Specific signal-carrying proteins or structures are shown below their corresponding category. The responses of cardiomyocytes and fibroblasts to these signals are shown to the right of each diagram. Red arrows next to each type of interaction notate whether an increase (up arrow) or decrease (down arrow) lead to the disease features presented.

Table 1

Models that can be applied to study myocyte-fibroblast interactions in the context of cardiac fibrosis and arrhythmias

Experimental models	Approaches /Types	Advantages	Limitations	References
2D Co-culture	Conventional	Simple, Direct view of cell-cell interactions	Random and small areas where cell- cell interactions interface; Difficulty in isolating cell types from cell-cell interactions	[14, 107, 108, 110]
	Transwell culture	Paracrine effects between two cell types	No direct cell-cell interactions	[109]
	Micropatterned (silicon combs, photolithography microfluidics)	Spatial control of cell interactions and larger areas where cell-cell interactions interface	Can physically isolate cell types from cell-cell interactions	[114–117]
3D Co-culture	Scaffold-based	Cell-cell interactions and Cell-ECM interactions can be assessed, Increased complexity of cell–cell interactions; Tissue-like structures; Measure physiology of tissue	Cell-ECM interactions dominate; Artificial ECM is needed; Difficulty in isolating cell types from cell-cell interactions	[120–123]
	Scaffold-free	Cell-cell interactions are enhanced; Increased complexity of cell cell interactions; Native ECM can form; Tissue-like structures; Measure physiology of tissue	Difficulty in isolating cell types from cell-cell interactions	[124]
Human Stem Cells	Human Induced Pluripotent Stem Cells	Human; Many cardiac disease models can be studied in the context of cell-cell interactions; more reflective of electrophysiological properties of human cells	Immature cardiac contractile apparatus; Difficulty in isolating pure fibroblasts	[125–129]
Heart Slices	Human diseased heart slices and Animal heart slices	Human; Native cell-cell interactions maintained in disease states; Measures physiology of tissue and more reflective of electrophysiological properties in intact heart tissue	Difficult to obtain controls for comparison; Difficulty in isolating cell types for cell-cell interactions	[131–133]
Mouse	Myocyte-specific gene targeting	Can study native myocyte-fibroblast interactions in vivo; Multiple cardiac-specific Cre models to temporally overexpress or ablate genes	Differences in cardiac electrophysiological properties in mouse versus humans	[16]
	Fibroblast- specific gene targeting	Can study native myocyte-fibroblast interactions in vivo	Few or inefficient fibroblast-specific Cre models; Differences in cardiac electrophysiological properties in mouse versus humans	[16, 134]

Experimental models	Approaches /Types	Advantages	Limitations	References
Computational models	Cellular/ionic model: (cell pairs; passive and active fibroblast model, ventricular myocyte model)	Can simulate different myocyte- fibroblast interactions; Can study the impact of manipulating myocyte-fibroblast interactions in the absence of disease; reproducible model system; Can be paired with biological data for comparisons; Can provide information on primary mechanisms	Not clear if findings are always reflective of the biological state *in vitro*; Limited number of parameters are included to reflect *in vitro* responses; Requires complex mathematical models and data analysis	[136, 138–143]
	3D whole heart models	Can simulate different myocyte- fibroblast interactions; Can study the impact of manipulating myocyte-fibroblast interactions in the absence of disease; Can generate human models; reproducible model system; Can be paired with biological data for comparisons; Can provide information on primary mechanisms	Not clear if findings are always reflective of the biological state *in vivo*; Limited number of parameters are included to reflect in vivo responses; Requires complex mathematical models and data analysis	[144–146]