Fibroblast-Myocyte Coupling in the Heart: Potential Relevance for Therapeutic Interventions

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Abstract

Cardiac myocyte-fibroblast electrotonic coupling is a well-established fact *in vitro.* Indirect evidence of its presence *in vivo* exists, but few functional studies have been published. This review describes the current knowledge of fibroblast-myocyte electrical signalling in the heart. Further research is needed to understand the frequency and extent of heterocellular interactions *in vivo* in order to gain a better understanding of their relevance in healthy and diseased myocardium. It is hoped that associated insight into myocyte-fibroblast coupling in the heart may lead to the discovery of novel therapeutic targets and the development of agents for improving outcomes of myocardial scarring and fibrosis.

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Keywords

scar; gap junction; cardiac electrophysiology

1. Introduction

Although cardiac function is governed by the contractile ability of cardiac myocytes, fibroblasts are one of the more numerous cell populations in the heart. While previously

thought to be electrical insulators without exception, we now know that fibroblasts may be electrically connected among themselves, and with myocytes.

Fibroblasts are key players in myocardial remodeling in response to disease and injury, proliferating (or being recruited from progenitors within and outside the heart) and differentiating into an activated phenotype that produces excess extracellular matrix (ECM) proteins, thereby helping to protect the heart from overdistension or rupture. While potentially of mechanical benefit, altered/increased fibroblast activity may contribute to cardiac arrhythmias, and may thus be a critical factor in disease progression. The purpose of this review is to examine the current knowledge on myocyte-fibroblast biophysical coupling in the heart, to sum up how this coupling changes in disease, to identify the steps needed toward a deeper understanding of these interactions, and to suggest how one might try to target these interactions for therapeutic benefit.

2. What occurs in native tissue

2.1. Cardiac composition and cellular function

While myocytes account for approximately 65–75% of cardiac volume, non-myocytes, such as fibroblasts or endothelial cells, constitute more numerous cell populations in the heart. Fibroblasts contribute, by some accounts, up to two thirds of total heart cells in rats [1] and humans [2,3], although mouse models may yield lower counts (20–30%) [4,5]. It is not currently known whether these variations reflect species differences (do large hearts, such as those of human [2,3], have higher fibroblast fractions?) or methodological challenges (counting approaches based on prior cell isolation [1,4,5] require identical 'relative survivals rates' of the different cell types – a precondition that is difficult to prove, while morphometric approaches are limited by their partial tissue 'snapshot' nature and the difficulty of extrapolating from two-dimensional [2D] section data to three-dimensional [3D] volumes, as cells with extended processes may traverse a given histological sectioning plane multiple times). Fibroblasts were initially thought to serve as a structural support system only, generating ECM into which cardiac cells are embedded, and thus providing the deformable skeleton of the heart. We now know that fibroblasts are of critical importance for a variety of cardiac functions, and that they are extensively involved in signaling in normal myocardium, and in remodeling/fibrosis during cardiovascular diseases. Of note, 'fibrosis' is a term that should not be confused with fibroblast density, as it is assessed by, and indicative of, elevated ECM levels, specifically of collagen.

In adult heart, myocytes are 'brick-shaped', with lengths in the order of 10^{-4} m and width/depth in the 10^{-5} m domain. Ventricular myocytes are organized into laterally reinforced laminae, 3–5 cells thick, that allow sliding of muscle layers ('sheetlets') during ventricular deformation. Long axis cell orientation (often, if incorrectly, referred to as 'fibre' orientation) varies from roughly −45° (relative to the horizontal plane of the ventricles and 'viewed' from the outside) in the epicardium, to +45° in the endocardium. Sheetlet structure is less easily described, and – depending on cutting angle of the virtual imaging or mechanical sectioning plane – could range from a transmural swirl (short-axis planes) to a fishbone pattern (long-axis planes) [6,7]. This fish-bone pattern is important for wall

thickening, which is associated with a more horizontal alignment of sheetlets during contraction [7].

Myocytes within sheetlets are connected in the axial direction at intercalated discs where the mechanical and most of the electrical junctions are found (Fig. 1A). Gap junctions, traditionally viewed as 'the' substrate for transmission of electrical signals between cardiac cells, are highly concentrated at intercalated discs, though they are also, if to a lesser extent, present at lateral cell contacts (Fig. 1B) [8,9]. In keeping with this, there is anisotropy in the speed of cardiac action potential (AP) propagation, being fastest along the main orientation of cardiomyocytes, slower in the fibre-normal in-sheetlet direction, and slowest normal to the plane of sheetlets.

Gap junctions consist of connexin (Cx) proteins. While connexin43 (Cx43) is the most abundant gap junctional protein in the working myocardium, other connexins including Cx40 and Cx45 are found in parts of the atria and in the pacemaker and conduction system [10–14]. All three connexin sub-types have been reported in cardiac fibroblasts [15–17].

Individual fibroblasts in the heart also form sheet-like structures [18], which explains why fibroblasts in 2D tissue sections almost invariably appear to have extended 'spindle-like' processes. They are interspersed between cardiomyocytes throughout the myocardium, and themselves interconnected by punctate connexin junctions [15]. Fibroblasts *in situ* have a surprisingly large surface area. One fibroblast, reconstructed in an electron microscopy (EM) study of the rabbit sinoatrial node, was measured to have 720 μm^2 of membrane surface area adjacent to a cluster of myocytes [18]. This only included one face of the cell membrane, and did not account the surface area of membrane folds or cell extensions not in direct apposition with the neighbouring myocytes. At the very least, the total surface area of this fibroblast would have been double the area it shared with the myocyte, *i.e.* in excess of 1,440 μm^2 [18]. This large membrane surface area highlights the ability of individual cardiac fibroblasts to interact, communicate, and signal with multiple homo- and heterotypic cells (the larger the membrane surface area, the larger the number of possible reaction and interaction sites), and to do so over significant distances. Such interactions may involve paracrine signalling, via release of growth factors and cytokines (reviewed in [19–21]), or occur via direct biophysical interactions through mechanical or electrical junctions (e.g. adhesion molecules, connexins, etc.), facilitating electro-mechanical transduction.

Defining 'the' cardiac fibroblast has proven difficult, as will be a common theme throughout this focused issue of *JMCC*. There is considerable variability in the developmental origin of cardiac mesenchymal cells [22]. The majority derive from the pro-epicardium [23]. Fibroblasts also originate from the epicardium by epithelial-to-mesenchymal transformation, followed by migration into atrial and ventricular walls where they differentiate into cardiac fibroblasts [24,25]. Additionally cardiac fibroblasts can originate from the cardiac endothelium, undergoing endothelial to mesenchymal transformation [26–28]. Although perhaps not frequent in normal homeostasis, other contributor pools to the cardiac fibroblast population include bone marrow-derived cells [8,9,10], hematopoietic cells [32], and mesangioblasts [33] (for review, see [34]).

This heterogeneity in cardiac fibroblast origins contributes to the ongoing challenge of finding suitable cardiac fibroblast markers that are specific and inclusive. In order to distinguish fibroblasts from other cardiac cell types, vimentin [35–37], discoidin domain receptor 2 (DDR-2) [38,39], transcription factor-21 (Tcf-21) [40,41], and fibroblast activation protein (FAP) [42–44] have been used – however, none are strictly specific, and each labels only a sub-population of the overall cardiac fibroblast population(s) [44–47].

2.2. Electrophysiological properties of cardiac fibroblasts

While the electrical integration of cardiomyocytes is associated with prominent clusters of connexin proteins – in particular Cx43 – at intercalated discs, fibroblasts in the normal myocardium of murine and rabbit heart express much smaller amounts of Cx43, Cx45, and Cx40 [48,49]. These proteins form punctate coupling sites between fibroblasts, whose functional relevance is ill-explored.

In the sinoatrial node of rabbit heart, *functional* heterotypic cell coupling of fibroblasts and cardiomyocytes has been illustrated by dye transfer studies (Fig. 1C) [15]. Apart from this, no firm functional data on heterotypic cell coupling in healthy heart has been reported. Structural coupling, for example, as indicated by co-localization of Cx proteins with membranes of heterotypic cells in the heart, is more common than was initially appreciated. This underappreciation may have been caused by the gap junction size, as this is near or below the detection limit of the early fluorescence techniques that gave rise to the text-book notion of cardiac Cx distribution and function [16].

Connective tissue, and by implication the fibroblast itself, was historically thought to be an electrical insulator. More recent studies have shown that this is not the only possible function of these tissues and cells. Patch clamp experiments have shown that the resting membrane potential of electrically discrete fibroblasts is between −10 and −50 mV *in situ* [50–52]. Fibroblasts in tissue have a high input resistance, with values in the GΩ region [50,51,53]. Since fibroblasts can be electrically interconnected to other fibroblasts *in vivo*, their membrane capacitance is difficult to quantify accurately. *In vitro*, values of 6–11 pF have been recorded [54,55], though freshly isolated fibroblasts are about an order of magnitude smaller than what has been documented *in vivo* (see above). In any case, their relatively low membrane capacitance, combined with a high membrane resistance, could make cardiac fibroblasts excellent long-distance electrical signal conductors.

Fibroblasts, while not an electrically excitable cell type, are able to *mimic* the AP of electrotonically coupled myocytes, as shown in dual-cell patch clamp experiments [50,52]. In this setting, cardiac myocyte AP generation drives membrane polarization in the passive fibroblast follower (helped by high resistance and limited capacity of the latter cell type). Fibroblasts further experience changes to membrane resistance and potential through mechanical perturbation, thus potentially assisting mechano-electric feedback in the heart [56].

2.3. Myocyte-fibroblast coupling

Exhaustive EM studies of gap junctions in the rabbit sinoatrial node have found extensive coupling between myocytes, small gap junction plaques between fibroblasts, but only a

single gap junction-like connection between a myocyte and fibroblast [18]. When fibroblast gap junctions are found in tissue, either by EM or by immunolabeling studies, they are small and punctate, making them difficult to find, and easy to mistake for background noise or artifact. Nonetheless, punctate Cx immunostaining is frequently found in myocardium between fibroblasts and at myocyte-fibroblast heterocellular connections [16]. These junctions may or may not be functional, as the presence of protein does not confirm function.

Tunneling nanotubes, which may involve (but do not require) connexins at their point of contact, can provide an alternative direct link for electrotonic coupling between myocytes and fibroblasts [57]. These thin (20–50 nm wide) membranous tubes can stretch between dendritic corneal cells as far apart as 300 μm *in vivo* [58], and they can propagate AP from cell to cell [59]. While evidence of myocyte-fibroblast coupling via nanotubes has been shown *in vivo* and *in vitro* [57], the electrophysiological function of nanotubes in the myocardium in health or disease has yet to be ascertained [34].

Ephaptic coupling – a non-electrotonic form of coupling – must also be considered as a possible mechanism of heterocellular electrical communication. Computer models have shown that ephaptic effects may be present in all areas of extracellular space, not just at the intercellular clefts of intercalated discs between myocytes [60]. More recent experimental evidence demonstrates this phenomenon in cardiac tissue [61], though there is still a need to understand how fibroblasts may be involved, if they are engaged at all [62].

Based on the arrangement of fibroblasts and myocytes in the heart, there are at least three conceptual modes in which these heterotypic cells could interact electrically. These modes may exist in healthy myocardium, as well as in various disease states.

First, in 'zero-sided coupling', fibroblasts are electrically not connected to myocytes, and instead are separating layers of functionally-connected myocytes, serving as an electrical insulator (Fig. 1D). This is presumably the most common form of fibroblast electrophysiological integration in native myocardium.

Second, 'single-sided coupling' may connect fibroblasts to an electrophysiologically homogeneous group of myocytes (Fig. 1D). In this case, fibroblasts would serve as a passive load, which could affect myocyte electrophysiological properties. Computer simulations over the past few decades have shown that functional myocyte-fibroblast coupling – by as few as 10–13 gap junctional channels [50] – could give rise to electrical source-sink relations, where fibroblasts may slightly depolarize the myocyte resting membrane potential, potentially accelerating pacemaker rate [50,63] or even having arrhythmogenic consequences [64].

Third, in 'double-sided coupling', fibroblasts would link myocytes that are structurally separated, and could serve as short (between muscle layers, Fig. 1D) or long range (in the case of multiple interconnected fibroblasts) conductors of electrical excitation. *In vitro*, this has been confirmed, as fibroblasts are able to electrotonically connect myocytes that are otherwise separated [65], passively bridging AP conduction over gaps of up to 300 μm [66]. If present at tissue and organ levels, this would have important effects on

electrophysiological behavior [67,68]. However, it is challenging to devise a conclusive experiment to test this possibility in native tissue, using traditional electrophysiological techniques. This is because the high membrane resistance of fibroblasts makes them excellent passive followers of an electrical source signal. While this is good for passive AP conduction, it is bad for experimental identification *in situ* – as well-coupled fibroblasts will be 'voltage-clamped by coupled myocytes' to passively display a myocyte-like AP.

3. What is different in a dish

Some of the above already points to differences between the *in vivo* and *in vitro* settings, such as increased electrical coupling of cells in a dish, compared to native myocardium. There is more.

Freshly isolated fibroblasts are small and rounded, without the membrane extensions and processes they possess while *in situ* (by the way: this reinforces the concern about cell quantification approaches that are based on prior isolation of cardiac tissue components). Their diameters are in the 7–9 μm range [54,69,70], and their membrane resistances are an order of magnitude higher than in fibroblasts assessed *in situ* [52,55].

Isolated atrial and ventricular fibroblasts 'carry through' to the dish some of their differences in morphology, with atrial fibroblasts being more elongated than ventricular fibroblasts [71]. When cultured, fibroblasts undergo a phenotype transition to myofibroblasts, induced, in part, by the large increase in substrate stiffness (from 10–15 kPa in native myocardium to GPa levels in plastic tissue culture dishes) [72–74]. This phenotype change involves increased expression of myofibroblast markers [75], including α-smooth muscle actin (α-SMA) [76], increased proliferation and migration, production of (and response to) transforming growth factor-beta (TGF-β) [77] and – increased Cx expression [78].

As a result of the Cx overexpression, electrical coupling of myocytes and fibroblasts via gap junctions *in vitro* is well-established. Indeed, synchronized contraction of distant cardiomyocytes, interconnected by fibroblasts only, has been observed *in vitro* in some of the earliest cell culture studies published [65,79,80].

Fibroblasts in culture have been found to express a number of ion channels and several currents have been recorded in patch clamp experiments *in vitro*. These include voltage gated K^+ channels with delayed rectifier currents and transient potassium currents in cultured rat and human cells [55,81–83]. Additionally, it is likely that K_{ir} is a primary determinant of resting membrane potential in both fibroblasts and myofibroblasts in culture, with changes in potassium concentration affecting not only membrane polarization, but also fibroblast proliferation and contraction [55]. Cardiac fibroblasts contain stretch activated non-selective cation channels [84–87]. In a rare report, $Na_v1.5$ expression was seen after long-term culture of human atrial fibroblasts [88], though this may have been a result of cell fusion [89] or exosome transfer [90], as no fast Na^+ currents are seen in freshly isolated fibroblasts – defined as a non-excitable cell population.

Genetically altering the expression of Cx and ion channels in fibroblasts *in vitro* has provided vivid illustrations of plausible effects of fibroblast coupling to myocytes [91]. Cx-

overexpression in fibroblasts has shown that coupling between myocytes and fibroblasts is not necessarily disruptive to conduction, and additional engineered ion channel expression in non-excitable cells can lead to conduction velocities close to those seen in pure myocyte cultures [92]. Interestingly, these genetic tools have shown potential as a therapy in post-myocardial infarction scars, as discussed below (section 5).

Fibroblasts cannot generate AP, but their membrane potential is responsive to mechanical stimuli. They may respond differentially to compression and stretch [93,94]. In cultures of rat ventricular fibroblasts and myocytes, increased heterocellular mechanical interactions via adhesion proteins caused conduction slowing in cardiomyocytes [95]. This suggests mechanical interactions may impair conduction as a result of increased mechanosensitive channel activation. Thus mechanical, in addition to electrical, coupling of myocytes and fibroblasts may affect cardiac electrophysiology.

4. What occurs in disease

Regardless of origin, if activated by injury, fibroblasts proliferate and increase production of ECM – one of the mechanisms believed to contribute to the domestication of atrial fibrillation [96]. Atrial and ventricular fibroblasts respond differently to disease, with atrial fibroblasts tending to generate larger amounts of ECM [71].

In the pressure-overloaded heart, resident cardiac fibroblasts of epicardial origin contribute to fibrosis [97], with a minor contribution from neural crest cells [98]. In contrast, studies of myocardial infarction (MI) suggest that, in addition to epicardially derived resident fibroblasts, bone-marrow derived cells contribute up to 60% of fibroblasts in the scar [29,32,99,100]. While TGFβ is an important regulator of cardiac fibrosis post-MI, fibroblasts are also activated by interleukin-1α (IL-1α), released by necrotic myocytes, which may be a critical contributor to the recruitment of extra-cardiac cells to the site of injury [101], compared to pressure-overload models.

In the ventricles, the increase in fibroblast number and ECM deposition after MI is thought to help prevent wall rupture. However, the changes that occur in the injury border zone– separation of myocytes by fibroblasts and ECM deposition, and lateralization of Cx43 (Fig. 1B) – affect AP propagation detrimentally [102–104]. Fibroblasts also remodel electrophysiologically post-MI, and show more hyperpolarized membrane potentials, increased outward current densities [105], and increased membrane resistance compared to fibroblasts isolated from normal hearts [53]. Further, the alteration in connexin profiles post-infarction can lead to increased fibroblast Cx43 expression [17,106,107]. Interestingly, decreased Cx43 expression in Cx43 knockout mice led to cardiac fibroblast activation and increased fibrosis in hearts, with a subsequent increase in arrhythmias [108].

The scar, as a whole, is formed largely by fibroblasts and ECM and is consequently considered to be electrically non-conducting. However, scars are more dynamic than this. Heterotypic fibroblast-myocyte coupling via Cx is often seen in the infarct border zone (Fig. 1B). Indeed, optical mapping of AP spread showed wave-front propagation into post-MI scar tissue, even after chemical ablation of any surviving sub-endocardial myocyte layers,

indicating a possible role for non-myocytes in passively conducting electrical signals [68]. The AP measured within the scar showed a reduced upstroke velocity and reduced amplitude – characteristics previously identified *in vitro* for cardiac fibroblasts coupled to myocytes [50,52] and compatible with computer modeling predictions [105,109]. Additional optical mapping studies identified a lack of calcium transients (a characteristic signature activity of myocytes) in the infarct scar [67]. This suggests that fibroblasts may serve as passive conveyors of AP waveforms in scar tissue. This could potentially explain trans-scar conduction recovery across atrial ablation lines seen in a majority of patients [110], and electrical coupling of recipient and donor tissue seen in about 20% of heart transplant patients [111].

That said, the above observations provide circumstantial evidence only, and functional heterocellular coupling in diseased heart has not been demonstrated with certainty. In the normal heart, functional *in vivo* data confirming heterocellular coupling has been shown by dye coupling in rabbit sinoatrial node [15]. Understanding the presence, extent, and regulation of myocyte-fibroblast functional coupling *in vivo*, how this coupling varies during development, by region, in various disease conditions, and whether it responds to medical interventions, is critical for development of fibroblast-targeting therapeutics. This is an area where 3D image reconstruction of scars may be incorporated into detailed computer models of conduction in disease [112,113], to assess plausibility of various concepts, and to devise testable hypotheses for experimental validation [114].

Beyond cellular resolution data, super-resolution microscopy may provide new insight into molecular and structural interactions important for heterocellular interactions within the heart. To date, super-resolution imaging has been applied to the structure of the intercalated disc and perinexus [61,115], the calcium handling machinery [116,117], and the Z-disc [118], some of which may be relevant for ephaptic coupling [61]. Super-resolution studies of cardiac fibroblast surface topography are a critical step towards better understanding of myocyte-fibroblast interactions in the heart.

Finally, while voltage-sensitive fluorescent dyes have allowed research into 'bulk behaviour' at the organ level, this approach does not allow attribution of observed behaviour to specific cell types. Such distinction could be achieved, however, by genetic targeting of reporter (or actuator) proteins to a specific cell population (optogenetics; see [119–121]). Recently, use of a voltage reporting protein, expressed under the α-myosin heavy chain (αMHC) promoter to target myocytes, or the Wilm's Tumor 1 (WT1) promoter to target non-myocytes in the heart, has been reported [122]. This study provided first evidence of AP conduction via non-myocytes within the scar border tissue.

5. Myocyte-fibroblast coupling as a therapeutic target?

Connective tissue remodeling is understood to contribute to arrhythmogenesis. Traditionally, this has ignored altered fibroblast electrophysiology, including potential electrical coupling to other cells including cardiomyocytes.

In vitro, fibroblasts are already well-coupled to co-cultured myocytes, and adeno-viral transduction to express additional ion channels and Cx43 in cultured fibroblasts may not necessarily give rise to drastic changes in electrophysiological behaviour of co-cultures [92]. However, the situation *in situ* appears different. Work from the Fleischmann group has shown that engraftment of Cx-expressing non-myocytes into post-MI ventricular scars of mice prevents arrhythmogenesis, and that this anti-arrhythmic action depends on the presence of Cx43 [123]. Additionally, injection of (inherently Cx43-expressing) non-myocytes that were transfected to over-express K^+ currents reduced automaticity and prolonged refractoriness in rat and pig hearts [124].

Another role of homo- and heterocellular Cx-coupling may be related to spread of injury across the cardiac syncytium. Thus, Cx43 mimetics – peptides that alter Cx function – have been found to improve outcomes after experimental MI. Examples include the peptides Gap26, Gap27 and Gap19, as well as αCT1 – a Cx43 c-terminal mimetic that interrupts Cx43-ZO-1 interaction. These peptides have been shown to reduce Cx hemi-channel function [125–127]. In experimental models of MI, Gap19 and Gap26 were shown to significantly decrease infarct size [128,129], and αCT1 was shown to reduce left ventricular dilation, maintain Cx43 at intercalated discs in the border zone, and decrease the number of inducible arrhythmias [130,131]. Though there is some understanding of the mechanistic effects of these peptides on gap junction intercellular communication, this has yet to be examined in the context of heterocellular coupling.

Delivery of bone marrow cells directly to infarcted myocardium as a therapeutic strategy has resulted in modest improvement of cardiac function at best. Further studies revealed that positive effects of cell injections on tissue repair were likely to be caused by paracrine mechanisms [132]. A different approach could exploit the fact that some non-myocytes are recruited to a healing infarct directly from the bone marrow [29,99,100]. These cells could provide a vehicle for delivering therapeutic 'payloads' to post-MI tissue [133]. Intrafemoral injection of lentivirus and adenovirus expressing GFP in mice has shown that cells within the bone marrow cavity can be efficiently transduced *in situ* [134,135]. Therapies of this type may be challenged by the fact that many more bone marrow cells would be transfected than those that home in to sites of cardiac injury [136]. This could be addressed, however, using 'auto-terminating' transfection strategies, or by the use of heterospecific antibodies, which have been shown to increase engraftment of intravascularly injected cells in the heart [137]. These antibodies are engineered to be specific for the antigens of two cell types. In this case, they first bind to the target cardiac cells, and then to specific antigens on the circulating cells. This strategy holds promise both with injected or bone marrow derived cells, as well as those recruited to the site of injury from inside the myocardium.

Interestingly, exosomes appear to account for some of the beneficial effects conferred by intracardiac cell injection [138,139]. In fact, exosomes administered intravenously are able to ameliorate cardiac function in a model of dilated cardiomyopathy [140], and a better understanding of exosome contents and their therapeutic potential is certainly high on the list of research priorities. As an exciting example, exosome cell surface proteins have been genetically engineered to display homing peptides, and this conferred targeting capability to cardiac myocytes and ischemic myocardium [141,142]. Of note, Cx43 is involved in the

communication between extracellular vesicles and mammalian cells [90], so exploration of exosome targeting to cardiac non-myocytes is a realistic prospect.

Clearly, there is a critical need for a better understanding of exactly how we should alter electrophysiology of scar tissue in the heart. Depending on purpose, answers may be at opposite ends of the spectrum. In the case of electrical connectivity, for example, ablation lines would ideally be made solidly insulating, while (at least small) cardiac infarcts might become less problematic if they did pass the wave of electrical excitation with minimal delay. So, in one scenario, one might wish to down-regulate nonmyocyte Cx expression, and in the other – up. We further need to understand how to improve the overall electrophysiological function of scar tissue without jeopardizing scar mechanics [143], how to deliver interventions in a spatially and temporally controlled manner, and how to grade them to achieve specific effects. All this depends, in part, on further insight into presence, distribution, functionality, and regulation of homo- and heterotypic coupling of cells in the heart.

6. Conclusions

Myocytes and fibroblasts interact in multiple ways, over a range of space and time scales, and in both normal and diseased heart. Molecular interaction substrates include electrical and mechanical junctions, and biochemical signaling makes significant contributions to myocyte-fibroblast cross-talk, including initiation of cellular phenotype changes in disease.

Presently, there is limited evidence of functional electrical coupling of myocytes and fibroblasts in the heart *in vivo*. Considering the heterogeneity of resident cardiac fibroblast populations, and the variability in cell types recruited to injured tissue, there is a need for detailed examination of the integrated myocardium in various disease states and regions in the heart, compared to steady state homeostasis. Clinical reports and experimental studies have provided evidence that scars may passively conduct electrical signals. Passive conduction is possible over fairly short distances only (signal amplitude attenuation), unless a supra-threshold stimulus reaches a 'repeater station', e.g. an island of surviving myocytes in post-MI scar tissue. Such active/passive/active chains of excitable and non-excitable cells have been engineered to successfully conduct excitation long-range, from atrium to ventricles [144,145]. The full extent of myocyte-fibroblast electrical coupling in the heart, the mechanisms of its regulation, and its importance in health and disease remain to be explored in detail. The growing number of experimental tools available to conduct cell-specific research raise the hope that we may be able to first, understand, and second, tweak scar properties to improve outcomes for patients with cardiac disease.

Acknowledgments

Abbreviations

2D	two-dimensional

3D	three-dimensional
αMHC	α-Myosin Heavy Chain
αSMA	α-Smooth Muscle Actin
AP	Action Potential
Cx	Connexin
Cx43	Connexin43
ECM	Extracellular Matrix
EM	Electron Microscopy
DDR2	Discoidin domain receptor 2
Tcf21	Transcription Factor-21
FAP	Fibroblast Activation Protein
IL-1α	Interleukin 1α
MI	Myocardial Infarction
WT1	Wilm's Tumor 1

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Highlights

- Fibroblasts (F) matter for cardiac structural integrity

- Furthermore, they are important biochemical and biophysical signaling hubs

- Biophysical signaling includes electric coupling of F to other F or to myocytes (M)

- F-M coupling may affect M excitability, refractoriness, and conduction

- The therapeutic potential of tuning F-M interactions requires further investigation
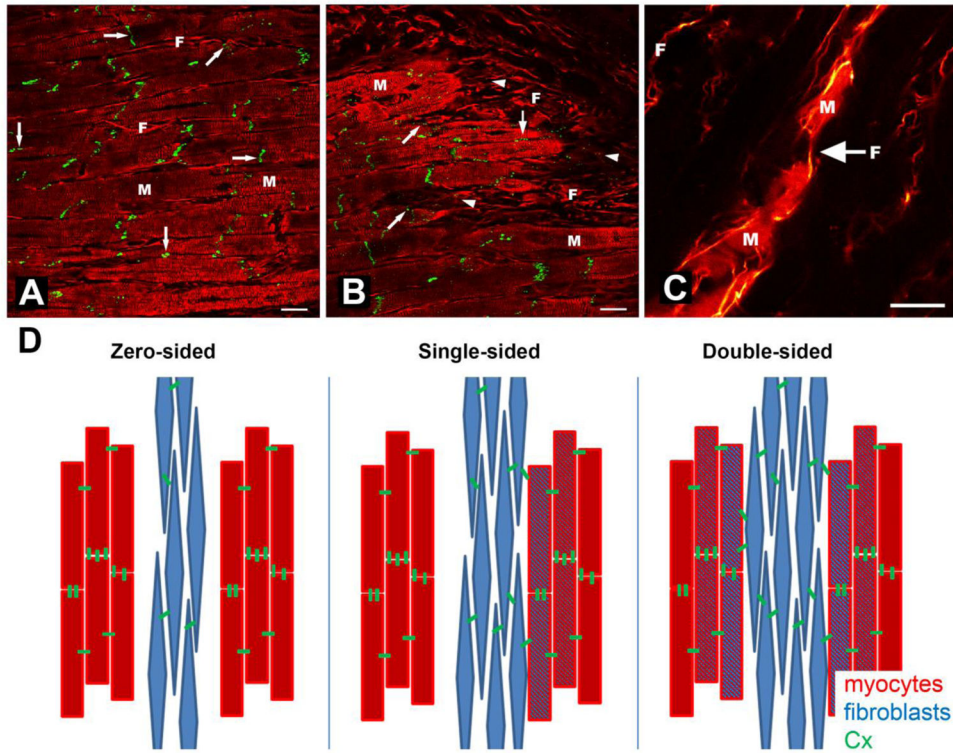
Figure 1.

Heterotypic intercellular connections in cardiac tissue. A) Cx43 (green) localized primarily at intercalated discs of myocytes in normal sheep myocardium (right-arrows), but also between lateral myocyte (M) contacts (down-arrows) and, occasionally, at points of heterotypic cell contact with fibroblasts (F; slanted arrow). B) Cardiac scar border zone, showing disturbed Cx43 distribution along M (increased lateralization; down-arrows) and abundant punctate presence in F (left-arrowheads), including at F-M junctions (slanted arrow). C) Confocal image of dye coupling by fibroblasts between groups of myocytes in rabbit right atrial tissue. D) Conceptual representation of myocyte-fibroblast electrotonic interaction scenarios via Cx proteins; for detail see section 2.3. Scale bars = 20 μm. From [17] in A and B with permission from the European Society of Cardiology, and [15] in C with permission from the American Heart Association.