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Structural and Functional Coupling of Cardiac Myocytes and Fibroblasts

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Abstract

Cardiac myocytes and fibroblasts form extensive networks in the heart, with numerous anatomical contacts between cells. Fibroblasts, obligatory components of the extracellular matrix, represent the majority of cells in the normal heart, and their number increases with aging and during disease. The myocyte network, coupled by gap junctions, is generally believed to be electrically isolated from fibroblasts *in vivo*. In culture, however, the heterogeneous cell types form functional gap junctions, which can provide a substrate for electrical coupling of distant myocytes, interconnected by fibroblasts only. Whether similar behavior occurs *in vivo* has been the subject of considerable debate. Recent electrophysiological, immunohistochemical, and dye-coupling data confirmed the presence of direct electrical coupling between the two cell types in normal cardiac tissue (sinoatrial node), and it has been suggested that similar interactions may occur in post-infarct scar tissue. Such heterogeneous cell coupling could have major implications on *in vivo* electrical impulse conduction and the transport of small molecules or ions in both the normal and pathological myocardium. This review illustrates that it would be wrong to adhere to a scenario of functional integration of the heart that does not allow for a potential active contribution of non-myocytes to cardiac electrophysiology, and proposes to focus further research on the relevance of non-myocytes for cardiac structure and function.

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The normal myocardium contains both myocytes and non-myocytes. Myocytes, the core of the contractile machinery of the heart, occupy the majority of the myocardial tissue volume, but account for only one third of the total number of cardiac cells [1]. Non-myocytes, consisting predominantly of cardiac fibroblasts, but also of endothelial and smooth muscle cells, account for the majority of cells in the myocardium.

Some cardiac regions, such as the sinoatrial node, are even richer in fibroblasts than the rest of the heart [2, 3]. Furthermore, fibroblast content increases with development and aging, as well as in a number of pathological conditions [4–8], and may contribute to the development of cardiac pathologies in senescent or diseased hearts.

Not only the content, but also the spatial organization of the two main cardiac cell types shows significant regional differences in the heart. In the ventricle, cardiomyocytes are arranged in sheets (about two to four cells thick), surrounded by a dense connective tissue network, and bridged both by branches of myocytes and fibroblasts [9–11]. In the sinoatrial node, pacemaker cells can be organized in clusters separated by connective tissue, or interspersed with fibroblasts. In all cardiac tissue areas, however, myocytes and fibroblasts are closely interrelated, with numerous anatomical contacts [11, 12].

Electrical communication between cardiomyocytes is via gap junctions [13, 14]. Whether similar communication between fibroblasts and cardiomyocytes occurs has been the subject of considerable debate [15, 16], and was only recently confirmed in native sinoatrial node tissue [17].

Both myocytes and fibroblasts are essential determinants of myocardial structure and function, and contribute to biochemical, mechanical and electrical signaling in the heart [12]. While the role of myocytes in cardiac electrical activity has been studied in great detail, that of fibroblasts is only starting to emerge.

Gap Junctions in Normal and Diseased Heart

Cell coupling in the heart is mediated by gap junctions, specialized channels which directly connect the cytoplasmic compartments of neighboring cells. Gap junctions are involved in many processes, such as growth, cell differentiation, apoptosis, and electrical and metabolic communication. In the heart, gap junctions are generally assumed to electrically couple cardiac myocytes, where they form low-resistance pathways that enable swift conduction of electrical impulses.

In the cardiovascular system, six different gap junctional proteins [called connexins (Cx)] have been identified: Cx37, Cx40, Cx43, Cx45, Cx46 and Cx50 (numbers represent the molecular mass in kilodaltons [18]). Different compartments of the heart express distinct connexin patterns that determine the particular conduction properties of cardiac tissue. Size, abundance and distribution of gap junctions are essential parameters that determine normal impulse propagation in the heart, crucial for a regular rhythm of cardiac contractions. Changes in gap junction expression, distribution and function are

often associated with conduction abnormalities and may promote arrhythmias [19–22].

The general distribution of gap junction proteins in different cardiac tissues has been widely studied. Cx43 is the main connexin in ventricular myocardium of all mammalian species, and is also present in atrial muscle and in the distal His-Purkinje system [23, 24]. Cx40 is abundant in atrial tissue and the conduction system (in particular in the sinoatrial and atrioventricular nodes) of numerous species, while it is absent in the ventricular myocardium [21, 22, 24–31]. Cx40, Cx43 and Cx37 form gap junctions in the aorta and pulmonary artery endothelium [24, 27, 32]. Cx45 is expressed in parts of the conduction system (sinoatrial node, atrioventricular node and bundle of His [30, 33]), but it is largely absent in the rest of the myocardium [34]. Cx50 has been found in the atrioventricular valves of rat heart [35] and Cx37 in the endothelium of the coronary vasculature [32].

There are divergent reports about the types of connexin proteins forming gap junctional channels in the sinoatrial node. Cx45 and Cx40 have been shown to be the main constituents in several species, including the rabbit [28–30, 36]. There are, however, many discrepancies in findings about the presence of Cx43: some reports conclude that Cx43 is absent in the sinoatrial node of most species, including the rabbit [30, 36–39], while others support the presence of Cx43 in the same region [40–42]. These differences may be species specific or linked to technical artifacts (reliability and specificity of antibodies are notorious sources of uncertainty).

Gap junctions undergo a remodeling process during many cardiac diseases [43, 44]. In the ischemic heart, two major abnormalities occur: downregulation of Cx43 in the well-preserved myocardium distant from infarction, and disruption of the normal Cx43 pattern, with dispersion over the myocyte cell surface at the infarct border zone [14, 45–51]. This gap junction remodeling, which occurs rapidly after infarction [52], promotes slowed and non-uniform conduction, which may increase cardiac susceptibility to arrhythmogenesis. In the hypertrophic heart, Cx43 is upregulated during the initial phase of compensatory hypertrophic growth, with a consequent increase in intercellular coupling, but is downregulated in the following chronic phase, causing a reduction in cell coupling and affecting cardiac conduction properties [49, 53–56]. Most investigations on gap junctional remodeling have focused on Cx43, the most abundant connexin in the heart, and only recent studies have extended the analysis to Cx40 and Cx45 [57–59]. Cx43 levels decrease while Cx40 increases in the human ventricle affected by congestive heart failure, where the Cx40 increase may have a compensatory role, improving cardiac activity of the compromised tissue [57]; Cx40 is downregulated and redistributed, with no changes in Cx43

levels, in the right atrium of patients with chronic atrial fibrillation [58], and Cx43 is downregulated and Cx45 is upregulated in the failing human left ventricle [59].

Cardiac gap junctions are conceptually associated with myocytes and assumed to couple homogeneous cells. A few studies have attempted to label muscle cells (using anti-desmin antibodies [36, 39–41]) or sinoatrial nodal cells (using the neurofilament marker or anti- α -smooth muscle actin [38, 39]), but no clear evidence of the localization of connexins between individual myocytes has been shown. The other large cell population in the heart, fibroblasts, has been largely ignored in such studies. Only recently, combined myocyte and fibroblast identification (using cell-type-specific antibodies: anti-myomesin for myocytes and anti-vimentin for fibroblasts), together with gap junction labeling, has provided a more accurate picture of gap junction distribution patterns and cell coupling in native cardiac tissue.

Myocyte-Fibroblast Coupling in Cell Culture

The ability of fibroblasts to contribute to *in vitro* impulse conduction has been known for almost 40 years. In the late 1960s, Goshima [60] and Goshima and Tonomura [61] reported synchronization of spontaneous cardiac activity in distant cardiac myocytes, interconnected by one or more fibroblasts, illustrating that myocyte-fibroblast heterogeneous electrical interaction occurs, at least *in vitro*.

The ability of myocytes and fibroblasts to form homogeneous and heterogeneous gap junctions *in vitro* has been addressed in direct electrophysiological investigations just over 20 years ago. Rook et al. [62, 63] showed that freshly isolated and cultured cardiac myocytes and fibroblasts readily form functional gap junctions, with single channel conductances (at room temperature) of 22 pS between fibroblasts, 43 pS between myocytes, and 29 pS between myocytes and fibroblasts (a value close to the conductance of Cx45 channels). Since cardiac fibroblasts have membrane resistances in the $G\Omega$ range, the action potential of a myocyte in a heterogeneous cell pair may drive the membrane potential of an electrically coupled neighboring fibroblast in a way that allows the fibroblast to display myocyte-like action potentials, even if the gap junctional coupling conductance is very small [62–64].

Fibroblasts possess a limited arsenal of ion channels [65], including stretch-activated ion channels [66]. Transfected fibroblasts expressing the voltage-sensitive potassium channel Kv1.3 can modify the electrophysiological properties of neonatal rat cardiomyocytes in culture [67], reconfirming that fibroblasts are electrically coupled to myocytes *in vitro*.

In 2003, Gaudesius et al. [68] extended the concept of cardiac fibroblasts as conductors of electrical excitation from the single cell level to longer distances. With a combination of functional (optical measurements of impulse propagation) and structural (immunohistochemical) studies, they revealed that cardiac fibroblasts were able to synchronize the electrical activity of cultured neonatal rat myocytes across gaps up to 300 μm in length. The molecular substrate for this *in vitro* coupling seems to be variable. Both Cx43 [62, 63, 69] and Cx45 [68] have been found at points of heterogeneous and homogeneous cell contact in neonatal myocytes and fibroblasts cocultured in monolayers.

This evidence confirms that myocytes and fibroblasts are able to form electrically conducting gap junctional channels, and that fibroblasts actively influence the electrophysiology of myocytes, *in vitro*.

Myocyte-Fibroblast Coupling in the Normal Heart

In vivo, the ability of fibroblasts to directly contribute to cardiac electrophysiology has been proposed since the early 1990s, and has remained the subject of extensive debate [15].

Early electrophysiological studies, conducted using double-barreled microelectrodes inserted into subendocardial layers of spontaneously beating rat right atria, revealed changes in the fibroblast membrane potential that correlated to the action potential in neighboring cardiomyocytes. This provided first circumstantial evidence for myocyte-fibroblast capacitative and electrotonic coupling *in vivo* (fig. 1) [64].

Functional proof of myocyte-fibroblast coupling *in vivo* is difficult to obtain by electrophysiological means. A drawback of *in vivo* electrophysiological studies is that they depend on the electrical identification of cell types (with no visual control), which becomes the more difficult the better a fibroblast is coupled to a myocyte, as the high membrane resistance of fibroblasts causes them to passively ‘mimic’ the electrical activity of the coupled cardiomyocytes (fig. 1b). Electrophysiological studies, therefore, remained inconclusive in the absence of histological proof of cardiac fibroblast-myocyte coupling *in vivo*.

Initial attempts to define the histological substrate for heterogeneous electrotonic interaction in the heart focused on a tissue that is particularly rich in fibroblasts, even under normal conditions, the sinoatrial node. Transmission electron microscopy studies of serial cross-sections yielded only ‘a single tiny gap-junction-like structure’ between a rabbit sinoatrial node myocyte and a fibroblast in a tissue volume assumed to contain 10^4 homogeneous myocyte-myocyte gap junctions, while extended areas of close myocyte-fibroblast

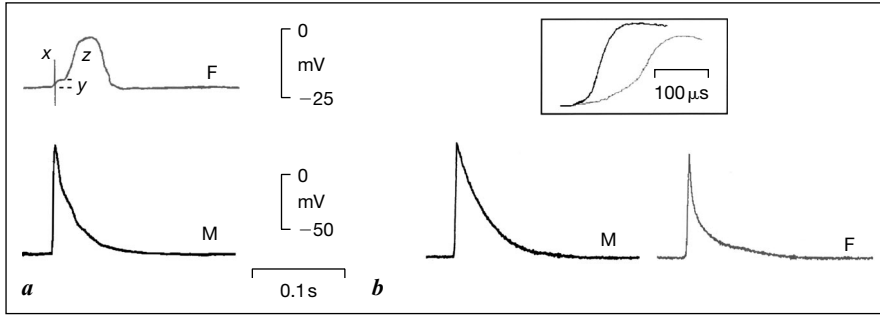


Fig. 1. In vivo myocyte-fibroblast electrical interaction. **a** Representative simultaneous recording of the action potential of a cardiomyocyte (M) and the membrane potential of a nearby ($40\ \mu\text{m}$ away) fibroblast (F) in the isolated right atrium of rat heart. The fibroblast is weakly coupled to neighboring cardiomyocytes, both capacitively [initial ‘stimulation artifact’ caused by the action potential upstroke in cardiac myocytes (x)] and electrotonically [membrane potential depolarization due to electrotonic interaction, probably via gap junctional channels (y)]. The deflection (z) is the mechanically induced membrane depolarization, typically observed in cardiac mechanosensitive fibroblasts during tissue contraction; upon electromechanical uncoupling of the tissue, components x and y remain, while z disappears in parallel with mechanical activity. **b** Simultaneous recording of the transmembrane potentials of a cardiomyocyte (M) and a nearby fibroblast (F) that is well coupled to surrounding cardiac myocytes. The action potential in M is representative for those generated by adjacent cardiomyocytes, including any that are in direct contact with F and that drive the electrotonically transmitted ‘mock action potential’ visible in F. The electrotonically induced delay in time to peak is about $100\ \mu\text{s}$ (insert). Modified from Kohl et al. [64], with permission.

membrane appositions were regularly observed [70]. The authors of that study interpreted this as evidence for a lack of heterogeneous cell coupling in native cardiac tissue.

Alternatively, heterogeneous cell coupling could occur via dispersed gap junctional channels in the abundant areas of heterogeneous membrane approximation, potentially forming structures that would not cluster densely enough to provide an electron-microscopically identifiable plaque, as suggested before in pig coronary artery [71].

Extended confocal microscopy studies of living rabbit sinoatrial node and right atrial tissue, involving the vital dye CellTracker[®] (CMFDA 5-chloromethylfluorescein diacetate), reconfirmed the presence of plentiful intimate membrane approximations between myocytes and fibroblasts in this tissue [72]. It has been proposed that these contacts provide a physical site for heterogeneous cell interaction [73], but the possible occurrence of gap junctional channels at these sites was not confirmed until recently.

In 2004, Camelliti et al. [17] used a combination of immunohistochemical and dye transfer coupling techniques, and confirmed the actual presence of functional homogeneous and heterogeneous gap junctions at numerous sites in the rabbit sinoatrial node. Cx40, mainly expressed by non-myocytes, was preferentially found at sites of contact between fibroblasts (fig. 2a, c, d), while Cx45, associated with both myocytes and fibroblasts, was involved in fibroblast, myocyte and myocyte-fibroblast coupling (fig. 2b-d, 3a). Functionality of homogeneous and heterogeneous cell coupling in the rabbit sinoatrial node was confirmed by dye transfer studies using Lucifer yellow (a gap-junction-permeable fluorescent probe), which revealed that fibroblasts form an extensive coupled network of cells able to form conductive bridges between myocytes that are themselves not in direct contact (fig. 3b) [17].

Sinoatrial node fibroblasts express both Cx40 and Cx45, but with a spatially distinct pattern, depending on the histomorphological environment. Fibroblasts in fibroblast-dominated regions devoid of myocytes preferentially express Cx40, which appears to be involved in homogeneous cell coupling. Fibroblasts that are intermingled with myocytes largely express Cx45, which would seem to be able to support both homogeneous and heterogeneous cell contacts (fig. 2). Cx43, Cx32 and Cx50 were absent from rabbit sinoatrial node [17].

While the above study shows myocyte-fibroblast coupling in the sinoatrial node, it is not yet known whether or not similar heterogeneous coupling may also occur in ventricular tissue. Ventricular myocytes and fibroblasts have been very recently shown to express both Cx43 and Cx45 in 4-day neonatal rat hearts [74], with Cx43 localized at points of homogeneous and heterogeneous contacts, and Cx45 mainly confined to fibroblasts and occasionally between the two cell types.

The hypothesis that fibroblasts may be a regular (and regulated) partner to myocyte coupling throughout the whole heart is further supported by preliminary immunohistochemical results showing potential heterogeneous cell coupling in rabbit and sheep ventricular tissue via Cx43 (fig. 4) [75]. Further research is underway to quantify such coupling in the ventricle or other cardiac regions, and to assess its functionality and possible relevance.

Myocyte-Fibroblast Coupling in the Diseased Heart

Several pathological states are associated with excessive growth of fibrous tissue, including collagen deposition, fibroblast infiltration and proliferation. Thus, pathological myocardium is often exceptionally rich in fibroblasts, which contribute to altering both structural and functional characteristics of the

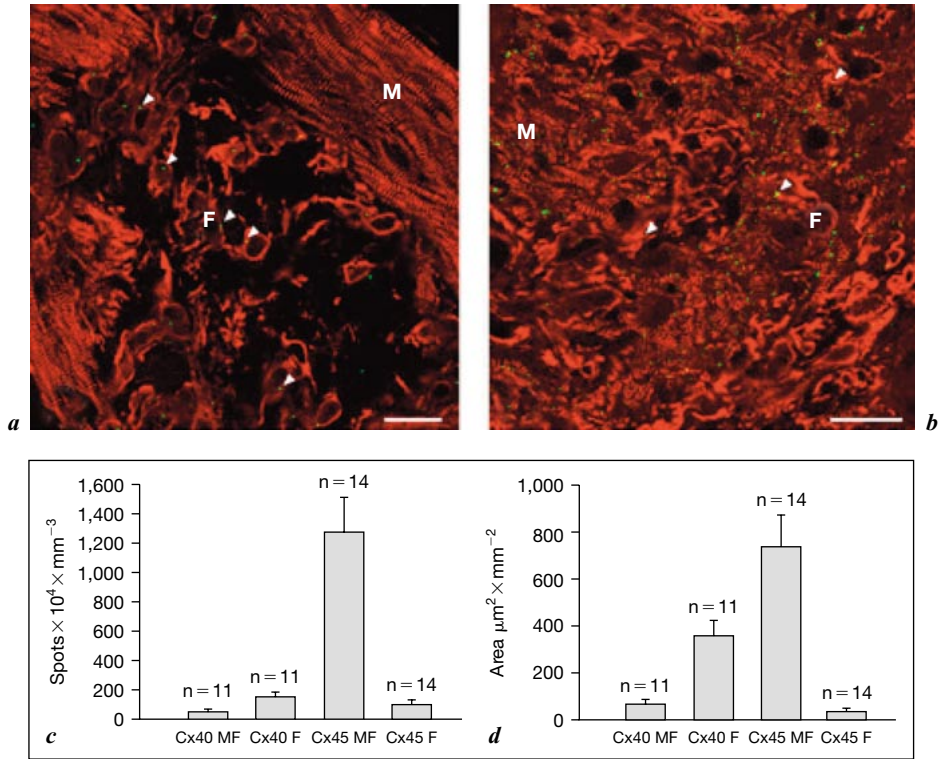


Fig. 2. Gap junctional expression patterns and levels in rabbit sinoatrial node. **a, b** Confocal images of central sinoatrial node tissue, immunostained with anti-myomesin to mark myocytes (red striated cells, M), anti-vimentin to mark fibroblasts (brightly stained solidly red cells, F) and anti-Cx40 (green dots, **a**) or anti-Cx45 (green dots, **b**). Fibroblasts in fibroblast-rich regions devoid of myocytes express Cx40 (arrowheads in **a**), while fibroblasts that intermingle with myocytes express Cx45 (arrowheads in **b**). Scale bars: 20 μm . **c, d** Quantification of Cx40 and Cx45 density (gap junctions per tissue volume, **c**, and area of gap junction fluorescence per tissue area, **d**) in intermingled myocyte-fibroblast (MF) and fibroblast-rich (F) areas. Cx40 density is significantly greater in F than in MF areas ($p < 0.001$). In contrast, Cx45 density is significantly greater in MF than in F regions ($p < 0.0001$). Both connexins have similar levels, in terms of spots per volume unit, in F areas (Cx40 F vs. Cx45 F in **c**), but in terms of gap junction fluorescence per tissue area, Cx40 is the most abundant connexin associated with fibroblasts (Cx40 F vs. Cx45 F in **d**; $p < 0.0001$). In MF regions, Cx45 is the predominant gap junction, while Cx40 is present at very low levels (Cx45 MF vs. Cx40 MF in **a, b**; $p < 0.0001$).

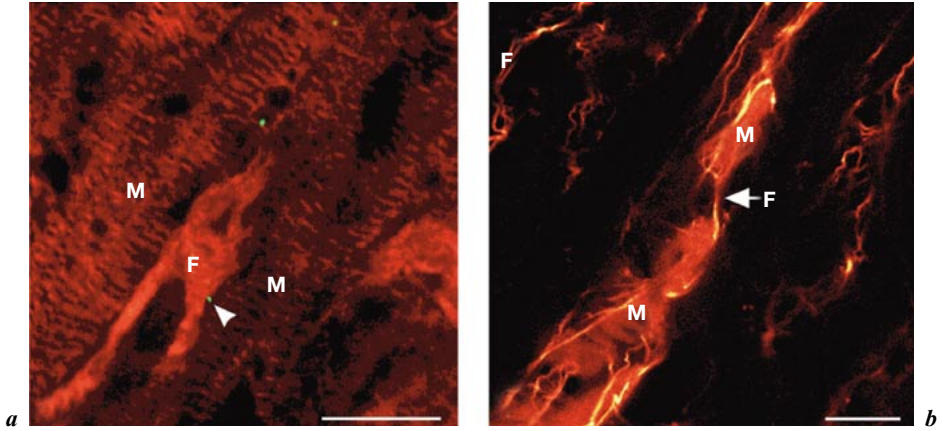


Fig. 3. Myocyte-fibroblast structural and functional coupling in rabbit sinoatrial node. **a** Confocal image showing myocytes (red striated cells, M), fibroblasts (brightly stained solidly red cells, F) and Cx45 (green dots). One of the Cx45 spots (arrowhead) in the given optical plane lies between a myocyte and a fibroblast. **b** Lucifer yellow spread through a heterogeneous chain of fibroblasts (F; thin, brightly labeled cells) and myocytes (M; larger 'orange' cells). Dye-filled myocytes form two groups interconnected only by dye-filled fibroblasts (arrow). Scale bars: 10 (**a**) and 20 μm (**b**). From Camelliti et al. [17], with permission.

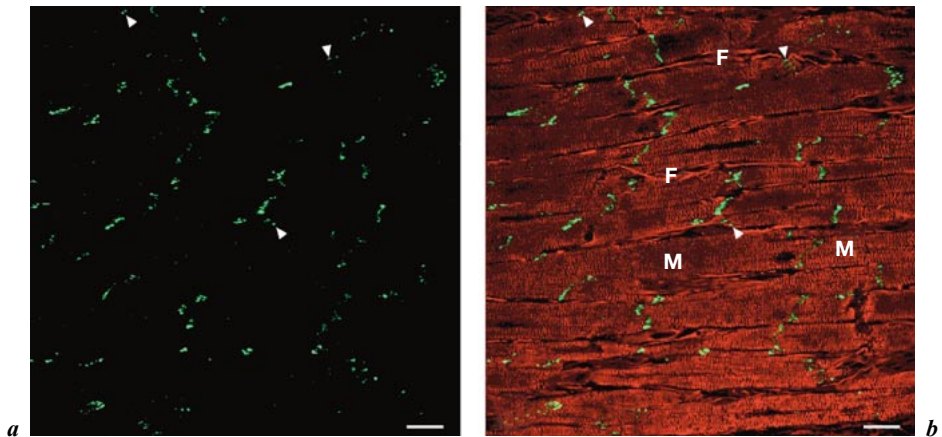


Fig. 4. Cx43 in sheep normal ventricular tissue. **a** Single labeling for Cx43 (green dots) shows intercalated disks, but also some dispersed punctate labeling (arrowheads), which would – in the absence of cell type identification, potentially be discarded as 'noise'. **b** Triple labeling for Cx43 (green), myocytes (striated red, M) and fibroblasts (red, F) reveals Cx43 not only in intercalated disks between myocytes (as expected), but also at points of contact between myocytes and fibroblasts (arrowheads). Scale bars: 20 μm . From Camelliti et al. [75], with permission.

diseased heart. Fibroblasts, coupled to other fibroblasts and/or to myocytes, may play an important role in the biochemical, mechanical and electrical behavior of the pathological heart and in the structural and functional remodeling.

A recent report highlighted extensive changes in gap junction distribution and cell coupling in a sheep coronary occlusion infarct model (from 12 h to 4 weeks after infarction [75]). Cx43, which is normally organized in intercalated disks between myocytes, undergoes dramatic remodeling after myocardial infarction, acquiring a punctate dispersed pattern along the surface of structurally compromised myocytes that intermingle with fibroblasts in the so-called 'infarct border zone'.

Fibroblasts in sheep infarcts (in contrast to previous observations in human and rat scar tissue [45, 76]) express Cx45 and/or Cx43, but not Cx40. They form gap junctions with fibroblasts and, possibly, other cells in the infarct [75]. Two spatially and temporally distinct fibroblast phenotypes were identified based on connexin patterns. Cx45-expressing fibroblasts appear in the damaged tissue within a few hours after infarction and reach their peak density after 1 week, while Cx43-expressing fibroblasts emerge later and continue to increase in number until at least 4 weeks after infarction [75]. Cx43- and Cx45-expressing fibroblasts were systematically found in close proximity to myocytes at the infarct border zone (fig. 5a). Further investigation is needed to assess functionality of homo- and (potentially) heterogeneous coupling, to quantify contacts, and to establish their role in the remodeling process after infarction.

Of note, islands of morphologically normal myocytes were regularly found inside the scar tissue, surrounded by tissue with high fibroblast density. Some of these myocyte islands were not detectably bridged via strands of myocytes to the healthy 'bulk' myocardium [75]. These viable myocytes form close contacts with the surrounding Cx43-expressing fibroblasts (fig. 5b), and it will be interesting to investigate whether and how they are functionally integrated with the normal myocardium.

One possible scenario would involve fibroblast-based connections. This concept is supported by the recent discovery of electrical impulse conduction from rabbit bulk myocardium into transmural scars 8 weeks after myocardial infarction. Interestingly, optically recorded epicardial action potential waveforms inside the scar region were similar in shape to cardiac action potentials, except for their somewhat reduced amplitude and upstroke velocity [77, 78]. In this regard, they were very similar to the intracellularly recorded activity of cardiac fibroblasts that are well coupled to a myocyte source, as seen in vivo (fig. 1b) [64] and in vitro [63].

The optically recorded action potentials in the scar could originate either from the remaining dispersed cardiomyocyte islands in the fibrous tissue (but this would require fibroblasts to act as conduction lines for their excitation), or

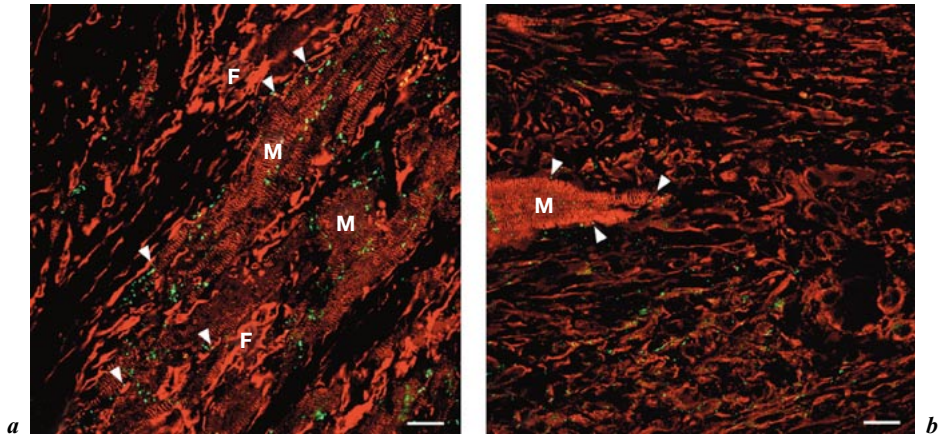


Fig. 5. Gap junction distribution and cell coupling in ventricular infarcts. Confocal microscopy sections of sheep ventricular infarct border zone (*a*) and mid-infarct scar tissue (*b*), immunostained with anti-myomesin to mark myocytes (red striated cells, M), anti-vimentin to mark fibroblasts (solidly red cells, F) and anti-Cx43 (green dots). Cx43 is expressed both by fibroblasts and myocytes, and is present at the interface between the two cell types (arrowheads), where it could potentially be involved in myocyte-fibroblast coupling. Scale bars: 20 μ m.

directly from cardiac fibroblasts (whose transmembrane potential may mimic cardiac action potentials). Both the fact that excitation spreads into the scar tissue and that the observed waveforms resemble those of cardiomyocytes (in the absence of significant numbers of surviving cardiomyocytes) are compatible with the notion that fibroblasts contribute to impulse conduction in this particular experimental model [79].

Relevance of Myocyte-Fibroblast Coupling

The structural, biochemical and mechanical relevance of myocyte-fibroblast interrelation in normal and diseased heart has been firmly established. The two cell types have been shown to be able to regulate cardiac responses to mechanical stimulation and pathological conditions, building on cross-talk that involves mechanical interaction and humoral factors released by the two cell populations [8, 80].

In the normal and diseased myocardium, fibroblasts form a highly coupled network [17, 75] that, at least in the sinoatrial node, is functionally linked to myocytes. Fibroblasts may, therefore, contribute to several chemical and biochemical processes, such as metabolite regulation, nutrient transfer, waste

removal and biochemical signaling, similar perhaps to astrocytes in the nervous system [81, 82]. In addition, the highly coupled fibroblast network in the diseased myocardium could also be involved in inflammatory responses, lesion spread, and myocardial remodeling, including regulation of cell proliferation and migration, extracellular matrix remodeling, and release of cytokines and growth factors after myocardial infarction [83, 84]. In diseased hearts, direct fibroblast-fibroblast and fibroblast-myocyte coupling could provide a substrate for progressive infarction via gap-junction-mediated bystander effects, where fibroblasts contribute to the spread of remodeling-related signals from the infarcted myocardium to surrounding tissue via gap junctions, potentially similar to the spread of neuronal damage via coupled astrocytes [12, 85, 86].

The relevance of the myocyte-fibroblast interrelation in cardiac electrical function has been, and still is, a controversial subject. This is caused, in part at least, by the difficulty in extrapolating from structural data and/or circumstantial evidence to actual functional relevance (thus far, functionality of coupling between cardiac fibroblasts and myocytes has been firmly established for rabbit sinoatrial node only). In addition, it is not always easy to confidently identify fibroblasts on the basis of morphological parameters (most labels are not strictly specific) or in situ cell electrophysiology (fibroblasts mimicking transmembrane potential dynamics of coupled heterotypic neighbors), and even the very concept of what a fibroblast actually represents is not uniquely defined: a specific cell type; a pluripotent cell; a transitory stage of cell development; or anything we cannot place into any other category?

Generally, fibroblasts have been considered to affect cardiac electrophysiology only passively. By forming electrically insulating septa between groups of muscle cells, in particular in aged and diseased sinoatrial nodes, or in areas of fibrosis and scarring, they act as passive obstacles for electrical excitation, contributing to discontinuous conduction, increased cardiac electrical heterogeneity and arrhythmogenesis [87, 88].

Both in vitro [63, 68] and in vivo evidence [17, 64] suggest that cardiac fibroblasts can be structurally and functionally coupled to other fibroblasts and myocytes via gap junctions. Such coupling would allow fibroblasts in vivo to influence cardiac electrical activity not only passively but also actively, by modulating electrical properties of cardiac tissue or by bridging gaps between excitable cells. The potential role of such electrical coupling varies in normal and diseased heart, in different cardiac tissues, and with age, and it could be particularly relevant in regions with high fibroblast content, like the sinoatrial node or infarct tissue.

Fibroblasts coupled to myocytes could act as current sink. By imposing an electrical load, they could induce regional shortening of action potential duration, arrhythmogenic excitability gradients, electrotonic depression of the tissue, and

contribute to slow conduction and unidirectional block, in particular in ischemic zones or, for example, in the atrioventricular node, where fibroblast content is exceptionally high.

Alternatively, fibroblasts coupled to myocytes could be involved in impulse conduction over short or long distances. As short-distance conductors, fibroblasts may interconnect groups of sinoatrial node cells separated by fibroblast aggregations, or layers of myocardial tissue separated by connective tissue and/or extracellular gaps in the ventricular wall. Here fibroblasts could provide electrical coupling that supports synchronization of the heterogeneous sinoatrial node, or contributes to the smoothing of electrical propagation in the cross-sheet direction of healthy ventricular tissue. As long-distance conductors, fibroblasts could bridge donor and recipient tissue after heart transplantation (in 10% of cases, such donor-recipient coupling manifests itself in patients [89]), or connect islands of surviving myocytes, found inside scar tissue, with the surrounding healthy myocardium. Here fibroblasts, via gap junctional coupling with other fibroblasts and with myocytes, could be responsible for the electrical integration of post-transplantation tissue across connective tissue barriers, for the impulse invasion of ventricular infarct scars, and conduction of electrical activity across the scar tissue. Such fibroblast bridges could have beneficial or detrimental effects. On one hand, they could provide a mechanism for electrical communication and synchronization of excitable tissue separated by non-excitable barriers, but on the other hand, the slow conduction, which occurs across long fibroblast inserts [68], could be arrhythmogenic.

Potential therapeutic treatments could involve changes in electrical coupling between myocytes and fibroblasts. It is by no means simple to predict whether an increase or reduction in coupling should be favored, as both might facilitate normal electrical propagation, or induce arrhythmia. Selective enhancement of myocyte-fibroblast and fibroblast-fibroblast coupling during cardiovascular diseases (produced, for example, via cardio-specific fibroblast connexin over-expression) would increase metabolic and electrical coupling across fibrotic or scar tissue and potentially avoid or repair cardiac conduction defects (such as fragmented or slow conduction, block, or sick sinus syndrome) in order to prevent arrhythmias [90]. On the other hand, uncoupling may be the preferred intervention, since this has been shown to limit necrosis and infarct size in ischemic hearts, probably by reducing lesion spread via gap-junction-mediated bystander effects [91, 92]. Reducing connexin expression after myocardial infarction, ideally targeted at cardiac fibroblasts, might limit cell-cell-mediated propagation of damage and thus reduce the extent of cardiac dysfunction, and preserve passive and active properties of the surviving myocardium.

Furthermore, fibroblasts are mechanosensitive: they respond to mechanical stimuli, such as those imposed by the contractile activity of the surrounding

myocardium, with changes in their membrane resistance and potential (component z in fig. 1a) [93]. By direct gap junctional coupling with other fibroblasts and/or adjacent myocytes, they could be involved in heart rate response to changes in the mechanical environment [72], or in the local depolarization and triggering of action potentials in connected myocytes, observed in cardiac scar models subjected to transient stretch [94].

In conclusion, the dogma of an electrically coupled network of cardiomyocytes, which is not connected to other cell types in the heart, can no longer be sustained. The extent, regulation and role of heterogeneous cell coupling in normal and diseased heart require further targeted research. This research needs to contain a strong element of *in situ* work, since *in vitro* gap junction expression and coupling differ significantly from (patho)physiological conditions. Projecting further ahead, therapeutic strategies that target fibroblasts and/or myocyte-fibroblast coupling could become an interesting avenue for medical intervention and, possibly, treatment of arrhythmias; the pharmacological and genetic tools for amending fibroblast proliferation and coupling have started to emerge [95, 96].

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