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Novel therapeutic strategies targeting fibroblasts and fibrosis in heart disease

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Abstract

Our understanding of cardiac fibroblast functions has moved beyond their roles in heart structure and extracellular matrix generation, and now includes contributions to paracrine, mechanical and electrical signalling during ontogenesis and normal cardiac activity. Fibroblasts have central roles in pathogenic remodelling during myocardial ischaemia, hypertension and heart failure. As key contributors to scar formation, they are crucial for tissue repair after interventions including surgery and ablation. Novel experimental approaches targeting cardiac fibroblasts are promising potential therapies for heart disease. Indeed, several existing drugs act, at least partially, through effects on cardiac connective tissue. This Review outlines the origins and roles of fibroblasts in cardiac development, homeostasis and disease; illustrates the involvement of fibroblasts in current and emerging clinical interventions; and identifies future targets for research and development.

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Cardiac mesenchymal cells, often referred to simply as ‘fibroblasts’, are among the most populous cells of the heart.[1, 2] Fibroblasts are also a cell type most conspicuously linked to heart disease, as they are associated with tissue remodelling involving ‘fibrosis’.[1, 3–6] Fibrosis contributes to the leading causes of sickness and death in the developed world, including the pathophysiological consequences of myocardial infarction (MI), in which myocytes are replaced by a collagen-rich scar; and hypertension, diabetes, rheumatic heart disease, hypertrophic cardiomyopathy and heart failure, which are associated with the patchy or diffuse appearance of excess fibrotic tissue. Surplus collagen, generated by activated fibroblasts, increases the stiffness of the myocardial wall and is linked to myocardial remodelling, thereby impeding normal systolic and diastolic mechanical function.

Focal scarring and diffuse fibrosis are independent predictors of the likelihood of developing electrophysiological disturbances resulting in cardiac arrhythmias.[2, 7, 8] Therefore, inhibiting or reversing fibrosis and its adverse consequences is an established target of many widely used clinical interventions for treating heart disease, including angiotensin converting enzyme (ACE) inhibitors, aldosterone antagonists, and statins (Table 1). These interventions may lead to a partial recovery of contractile function via a process known as ‘reverse remodelling’, and thereby delay the progression towards heart failure.

The individual and societal costs of heart diseases linked to fibrosis are staggering.[9] In the case of ischaemic heart disease alone, the American Heart Association reports that each year approximately 635,000 Americans are hospitalized or die from their first MI. More than half survive, but each year around 280,000 patients will go on to suffer subsequent coronary events, leading to further loss of cardiac muscle and additional deposition of non-contractile and potentially arrhythmogenic fibrotic tissue. The direct costs arising from ischaemic heart disease in the USA are in excess of $180 billion dollars per year. Heart failure after MI represents a further annual burden of $20–40 billion dollars.

There is a strong clinical and economic case, therefore, for developing new and effective approaches to targeting cardiac disease processes linked to fibroblast function. This Review will consider the origin and roles of fibroblasts, illustrate their involvement in current and emerging clinical interventions, and identify future targets for basic and applied heart research.

Fibroblasts and adult myocardial homeostasis

In the healthy heart, fibroblasts are the main cell type involved in formation and maintenance of connective tissue.[1, 3, 10] Cardiac connective tissue consists of cellular and acellular components, with the acellular extracellular matrix (ECM) providing a flexible scaffold for individual myocytes and for the heart as a whole.[11] The highly organized collagen-rich meshwork, laid down by fibroblasts, stabilizes the myocardial wall and supports force transmission, while allowing for intricate patterns of tissue deformation.[12] This fibrous skeleton also provides regions that act as barriers and supports, for example establishing myo-fascial planes between sheetlets of muscle, reinforcing conduits for blood vessels,

separating electrical activation of the atria and ventricles, or insulating bundles of the cardiac conduction system.

The fibrous skeleton of the heart also provides a substrate for tissue-engineered repair.[13] In these experiments, perfused rodent hearts were decellularized, leaving the ECM scaffold, including the ECM surrounding the coronary vascular tree, intact. This ECM scaffold was then repopulated with neonatal myocytes, which – based on the available structural cues – settled to recapitulate a beating, heart-like organ. Whilst regeneration of a mature heart *in toto* by this approach remains a distant prospect, initial clinical applications of this method could include use of decellularized ECM patches for surgical repair.[14] These cardiac ECM scaffolds can be repopulated with induced pluripotent stem cells (iPSCs) – raising the prospect of patient-specific reparative therapies.[15] Conceptually, this builds on breakthroughs, over the past 10 to 15 years, in the pre-clinical and clinical application of decellularized allo- and xenografts for heart valve repair and replacement,[16] including the limitations and challenges associated with the approach.[17]

In addition to the maintenance of connective tissue, cardiac fibroblasts express a wide array of paracrine factors and cytokines and have complex biochemical and biophysical interactions with myocytes, thereby influencing development, growth, and functional adaptation of muscle cells.[1, 3, 5] Paradoxically, given the aforementioned role of fibrous tissue in insulation, there is evidence for direct electrical communication via heterocellular connexin (Cx) junctions between fibroblasts and myocytes[18–20]– interactions that may well have implications for the modulation of electro-mechanical activity of heart muscle in health and disease.[2, 7, 21] Thus, fibroblasts form physiological signalling hubs whose relevance extends well beyond classic roles in the generation and maintenance of ‘mechanically connective, yet electrically divisive’ tissue.

Developmental origins of fibroblasts

One of the impediments to developing new mechanistically based strategies to target fibroblast function in disease has been an incomplete understanding of their multi-lineage derivation and potential regulatory interactions between mesenchymal cells of differing origin. A characteristic feature of fibroblasts is the ability to generate ECM; however, this functional capacity is frequently left unconfirmed when classifying non-myocytes *in vitro* or *in vivo*. Nonetheless, considerable progress has been made over the past 25 years, and the associated new insight is beginning to affect our understanding of the normal and diseased heart (Fig. 1).

The pro-epicardium – a sprout-like cluster of extra-cardiac cells that first comes to prominence at the looped-tube stage of cardiac development[29, 44] – provides the source for a thin mantle of epicardial epithelium that progressively envelopes the embryonic heart.[22, 44] Epithelial-mesenchymal transition (EMT) at the epicardium is the largest initial source of resident interstitial cells (Fig. 2).[29, 45, 46]

Recent data suggest that the pro-epicardium itself is an evolutionary derivative of the primordium of an ancient pro-nephric glomerulus,[47] suggestive of interesting phylogenetic links between fibrotic processes in kidneys and heart. Paracrine signalling by fibroblast

growth factors (FGFs), bone morphogenetic proteins, retinoic acid, various members of the transforming growth factor β (TGFβ) family,[4, 48–50] as well as transcriptional activators including Wilms tumor 1 (WT1) protein,[51] and T-box proteins-18 and 20,[52, 53] have been identified as early regulators of epicardial EMT. These proteins affect pathways including canonical and non-canonical Wnt signalling and hedgehog and retinoic acid signalling, which have downstream effects on gene programmes that regulate intercellular interactions, actin dynamics and cell motility. Following invagination of mesenchyme from the embryonic epicardium into the myocardium, various signal transduction and regulatory molecules, including TGFβ,[54, 55] FGF,[56] platelet derived growth factor (PDGF),[57] proto-oncogene tyrosine-protein kinase,[55] rho kinase,[58] integrins,[59] popeye domain-containing genes such as Bves,[60, 61] serum response factor,[62] transcription factor 21 (Tcf21),[63, 64] and myocardin-related factors[65] govern the transition of epicardium derived cells (EPDCs) into cells with more mature fates, including fibroblasts.[5, 6] There is also evidence from studies in *Xenopus laevis* that Tcf21 functions as a transcriptional repressor to regulate proepicardial cell specification and the correct formation of a mature epithelial epicardium.[64]

Pericytes form a distinct cohort of mesenchymal cells that reside at perivascular locations.[4] Pericytes can express stem cell markers (e.g., Oct4, Sox2)[66] and may be fibroblast progenitor cells that are capable of self-renewal and differentiation into more mature mesenchymal phenotypes. There are unresolved questions as to whether pericytes contribute to cardiac fibrotic remodelling.[67] Prominent fibrosis around blood vessels is a feature of many diseases of the heart, and there is evidence for phenotypic variance between quiescent and activated fibroblasts at perivascular and interstitial loci.[3, 4]

Mesenchymal cells can also arise in the embryonic myocardium through endothelial-mesenchymal transition (EndoMT) of endocardial endothelial cells,[68] as well as from migratory populations of neural crest cells (Fig. 2).[69] Cells of endocardial origin primarily contribute to the developing inlet valves, whereas neural crestderived cells appear to be largely confined to the connective tissue of the great vessels and, possibly, the cardiac outflow tract.[70] Minor populations of fibroblast-like cells that originate from these sources are also resident in homeostatic myocardial tissues. Notable in this respect are neural crest-derived mesenchymal cells in the atria[71] and around proximal components of the cardiac conduction system.[72]

The tissues of the embryonic heart are thus subject to waves of immigration of mesenchymal progenitors from heterogeneous sources, with each of these different populations demonstrating some specificity in their spatiotemporal and, possibly, functional assignments in the developing organ.

Fibroblasts in the adult heart

Myofibroblasts—Fibroblasts are changeable in character. A pertinent aspect of this phenotypic variability is the transition of quiescent fibroblasts, via a proto-myofibroblast intermediary stage, into activated fibroblasts or myofibroblasts (Fig. 2).[1, 3, 5, 6] Pathways involved in the conversion of fibroblasts into myofibroblasts are thought to include TGF[73] endothelin-1,[6, 74] angiotensin II (Ang-II),[75] and Wingless-related integration site (Wnt)/β-catenin signalling.[3, 6] The gap junction protein connexin 43 (Cx43) has been found to

regulate TGFβ signalling in a non-channel-dependent manner,[76] and knockdown or over-expression of Cx43 inhibits or potentiates TGFβ-induced myofibroblastic phenoconversion, respectively.[77] Other signal transduction pathways implicated in regulating phenotypic conversion of quiescent fibroblasts into myofibroblasts include the peroxisome proliferator-activated receptor γ, JNK and Akt signalling pathways; some effectors of these pathways are also downstream of TGFβ signalling.[1, 5] The cell membrane protein caveolin-1[78] and a number of proteins mediating interactions between cell membrane receptors and the ECM (so-called matricellular proteins), including thrombospodins,[79] SPARC,[80] periostin,[81] tenascin-C,[82] and extracellular matrix-associated proteins (named CCN as an acronym derived from the first three proteins discovered in this family)[83] are also involved in regulating myofibroblast transdifferentiation. Whilst their contribution to heart function is evident, the specific roles of each of the matricellular proteins remain poorly characterized, but probably include the generation of a conducive mechano-environment, effects on directed migration, stress fiber formation and the production and/or activation of signalling factors, such as TGFβ1, that promote the conversion of fibroblasts to myofibroblasts.[84]

Many of the pathways governing the transition of fibroblasts to myofibroblasts are mechano-sensitive[85–87] and potentially involve stretch-activated ion channels and changes in cellular calcium handling.[88–90] Myofibroblasts are able to generate mechanical force, which is important for scar contraction and remodelling.[91] This is a consequence of upregulation of proteins involved in coordinated force- and tension-generation, including α smooth muscle actin (αSMA), myosins and junctional molecules such as cadherins and Cx43. These cells also have important roles in the active secretion and turnover of collagen and other ECM molecules[10] and are responsive to hypoxia, cytokines and signalling molecules associated with the inflammatory milieu, such as TGFβ, tumor necrosis factor α, interleukin-6 (IL-6), and IL-2.[1, 3, 5, 6, 92] Activated fibroblasts are a common component of granulation tissue throughout the body, typically peaking in density at 4–6 days post-injury. An interesting difference between wound healing in skin *vs.* heart is that myofibroblasts appear to persist (for up to 17 years!) in cardiac scars.[93] The mechanism for their preservation in healed post-MI scars may be related to the interplay between endothelin, angiotensin and TGFβ signalling. Maintenance of myofibroblasts in the scar post-MI is thought to confer mechanical benefits: murine strains with high densities of myofibroblasts in healed infarcts showed improved ventricular function and reduced propensity for scar expansion and ventricular dilation.[94]

Resident and non-resident mesenchymal cell populations—The heterogeneous origin and phenotypic variability of fibroblasts pose technical challenges for experimental cell identification. Expressed proteins, including discoidin domain receptor tyrosine kinase-2 (DDR2), periostin, vimentin, fibronectin ED-A, WT1, PDGF receptor, TCF21, αSMA and CD90/Thy1, are commonly used to identify fibroblasts and myofibroblasts.[4] However, these markers (including some whose naming wrongly implies fibroblast-specificity, e.g. so-called fibroblast-specific proteins such as FSP-1)[95] may not be uniquely or continuously expressed and they do not, therefore, provide a means for reliable tracking of the differentiation history of mesenchymal cells.

Basic studies of cell fate in chick[29] and mouse[4, 96] embryos using genome integrating virus and transgenic lineage markers, respectively, were key to the demonstration that early populations of cardiac fibroblasts are derived from the pro-epicardial organ. More recently, genetic fate-mapping tools have contributed to new insights into fibroblasts in adult heart and disease. Using Cre-expressing lines and a collagen1α1-green fluorescent protein (GFP) fusion reporter, the majority of fibroblasts in a mouse model of fibrosis, induced by pressure overload, were shown to be derived from cells resident in the heart, mainly from epicardial sources.[43] Concurrently, a study in which mice expressing Tbx18-Cre, Tie2-Cre and Pax-3-Cre were used to determine the origin of fibroblast lineages came to a similar conclusion – although a minor contribution by neural crest-derived cells was also noted.[71]

A twist was recently added to this story. Cre-LoxP technology was used to track epicardial- and bone marrow-derived mesenchymal cells,[97] and although pressure overload did indeed trigger fibroblastic differentiation predominantly from resident EPDCs in these experiments, collagen deposition and scar formation following MI was determined by interactions between cells originating from both the bone marrow and the pro-epicardium. This study further suggested that regulatory interactions between the two different mesenchymal populations determined the transition to pathological levels of fibrosis – though it did not provide data that directly supported this interesting hypothesis. Notably, this report used WT1-Cre, which, because of its activation at the earliest stages of pro-epicardial differentiation, tags of a range of cardiac cells, largely with non-myocyte phenotypes.

The conclusions of this work,[97] however, support earlier studies that detected hematopoietic contributions to mesenchymal cell populations in the heart, including to post-MI scar formation.[36, 98] These reports were based primarily on chimeric mouse models, wherein genetically tagged (e.g., GFP) bone marrow cells were transplanted into irradiated (bone-marrow incompetent) animals. The tag was then used to trace the fate of marked cells. Using this method, it was estimated that 28–57% of fibroblast-like cells in post-MI scar tissue were of bone marrow origin, with a third of these cells adopting a myofibroblastic phenotype.

In a noteworthy extension of this experimental approach, hematopoietic stem cells were evaluated in a clonogenic manner *in vivo* by repopulating lethally irradiated murine recipients with a single GFP-marked bone marrow-derived stem cell clone.[98] Following surgically induced MI, clonally derived GFP-expressing cells were found to densely populate post-MI scars. These cells were of fibroblastic morphology and persisted in scar tissue 30 days post-MI, a time point at which the inflammatory response was already largely resolved.

Bone marrow-derived, or myeloid fibroblasts (also known as fibrocytes) are recruited to injured loci as monocytes from the blood via the stromal cell derived Factor 1α/chemokine receptor type 4 signalling axis. They undergo a further transition to $CD45^+$ mesenchymal cells under the influence of cytokines, including IL-6 and monocyte chemoattractant protein-1 (MCP-1).[99] Myeloid fibroblasts contribute to non-adaptive cardiac fibrosis and adverse remodelling, particularly post-MI and during ischaemic cardiomyopathy, heart failure and aging.[99, 100] In this context, TNF signalling, which regulates the transition of macrophages from an M2 phenotype (immunosuppressive, pro-angiogenic macrophages) to

fibroblast-like cells, could be particularly relevant, as has been shown in models of heart disease.[101]

Finally, endothelial cells in the adult heart may undergo EndoMT and contribute to fibrosis after myocardial injury.[102] In this process, endothelial cells lose their endothelial identity and acquire mesenchymal or myofibroblastic markers, such as αSMA expression and type I collagen secretion. Similar to EMT, EndoMT can be regulated by TGFβ.[102] Interestingly, recent studies suggest that this process can also be reversed, and mesenchymal cells can generate coronary vascular endothelial cells by mesenchymal-endothelial transition in a p53-dependent manner.[103]

Thus, available evidence suggests that three main cell populations contribute to fibrosis after injury or in pathologic conditions in the adult heart: resident fibroblasts derived from EPDCs, mesenchymal populations originating from circulating precursors and cells that have undergone EndoMT (Fig. 2). There may also be a fourth minor contribution from neuroectoderm.[71] EPDCs seem to be the major contributor to fibroblast cell numbers overall, but ongoing studies may well uncover disease-specific responses by different lineages, including regulatory interactions between the different mesenchymal populations that vary according to pathology.

Work on targeting or using mesenchymal cells for treating cardiac disease is broad and intensive.[104] Key foci of current translational research in this context are on regenerating heart muscle lost to disease by reprogramming fibroblasts to trans-differentiate into cardiomyocytes, and on reducing fibrosis or the extent of pathologic scarring that occurs in response to injury and/or disease (Box 1)

Fibroblast reprogramming in cardiac repair

Fibroblast reprogramming falls into the burgeoning field of regenerative medicine. Whereas the adult mammalian heart repairs by forming a scar, amphibians and fish can regenerate injured myocardial tissue.[105, 106] There have been numerous tantalizing hints that therapies could be developed which recapitulate or simulate regenerative processes similar to those occurring in lower vertebrates, most recently from studies of reparative responses in newborn mice.[107, 108]

The rationale for exploring whether fibroblasts can be reprogrammed into myocytes as a cardiac regeneration strategy can be traced back to the Nobel prize-winning discoveries of Yamanaka and colleagues, who reported the generation of iPSC from somatic cells by retrovirus-mediated overexpression of a combination of four transcription factors – Oct4, Sox2, Klf4 and c-Myc.[37] Subsequently, it was shown that forced expression of lineage-specific factors could reprogramme cells into defined phenotypes, without reverting to an intervening stem cell.[109] In cardiac biology, Gata4, Mef2c, and Tbx5 (collectively referred to as GMT), are sufficient to prompt the trans-differentiation of murine cultured fibroblasts into cardiomyocytes with early ontogenetic traits.[42] The GMT cocktail –subsequently extended by adding a fourth transcription factor, the heart and neural crest derivatives gene 2 (Hand2) – improved cardiac function in a murine model of MI.[110, 111]

In parallel, micro-ribonucleic acid (miRNA) was shown to reprogramme cardiac fibroblasts into cardiomyocytes *in vitro* and *in vivo*.[112] Priming of EPDCs with the actin-binding peptide thymosin-β4 also triggers trans-differentiation of these cells into myocytes – albeit at very low frequency[113] and potentially not in clinically relevant settings.[114]

The path to cardiac reprogramming has not been smooth. In a replication study, the GMT-induced conversion was relatively inefficient.[115] This work also indicated that the electrophysiological phenotype of converted fibroblasts might not align with that of cardiomyocytes. Methodological differences between studies may underlie variable observations. For example, vector-dependent imbalances in protein expression of the G, M, and T factors have been proposed as one reason underlying study-to-study variation.[116] A further impediment to translation may come from the finding that human cardiac fibroblasts appear to be resistant to reprogramming by GMT, though may convert at low efficiency to immature beating myocytes if GMT is combined with Hand2, myocardin and two muscle-specific miRNAs: miR-1 and miR-133.[117]

Ongoing work on fibroblast reprogramming has focused on improving its reliability, safety and efficiency. These efforts include the optimization of the stoichiometry of factors in the reprogramming cocktail;[116] identification of combinatorial miRNA treatments;[118] development of delivery methods other than genome-integrating retroviruses;[119] and attempts to generate specific cardiac phenotypes, including atrial, ventricular and pacemaking cells,[120] with the long-term aim of producing cells with adult-like structural and functional properties. In addition, manipulation of cell transduction pathways including those associated with TGFβ[121] and Akt1 signalling[122] could increase the efficiency of cell reprogramming. Enhancing Akt1 activation notably increased the efficiency of fibroblast to myocyte conversion *in vitro*.[122] Interestingly, thymosin β4, which activates Akt, has been reported to enhance function post-MI in the presence of GMT reprogramming.[110]

Novel drugs and biologics

Partial recovery of disease-induced changes in structural and functional tissue properties (reverse remodelling) is a target of clinical interventions,[123] in part because fibrosis is an independent predictor of arrhythmogenesis and sudden death in patients.[124, 125] Several established pharmacological therapies that are known to reduce arrhythmias or progression of heart disease affect connective tissue, including ACE inhibitors, aldosterone antagonists and statins.[1, 3, 6] Novel cardioprotective therapies are being developed that preserve myocardial muscle, thereby inhibiting its replacement by scar tissue in the acute phase following cardiac injury. In addition, interventions, including cell therapies, may provide both cardioprotection and enhance the ongoing wound healing response of damaged hearts. Third, pharmacological approaches are also being developed to target chronic pathologic processes that lead to the accumulation of fibrotic tissue and heart failure. These are discussed in detail below.

Cardioprotection—The single most important contribution to reducing the fibrotic burden in ischaemic heart disease in the last 25 years has come from the advent of reperfusion procedures such as angioplasty and antiplatelet and antithrombotic medications, which

restore blood flow to ischaemic myocardium in the immediate aftermath of MI.[126] Myocyte viability is critically dependent on the availability of sufficient oxygen and thus swift re-establishment of coronary vascular flow following MI increases the survival of myocardial cells, thereby reducing post-infarction fibrosis. Whilst revolutionizing the critical care of MI patients, the generation of reactive oxygen species associated with reperfusion can itself cause damage to the heart, known as ischaemia-reperfusion injury, resulting in diminished contractile function and increased risk of arrhythmia.[127]

Ischaemic pre-conditioning, in which short episodes of hypoxia reduce infarct size upon subsequent MI,[128] has inspired the search for therapeutic approaches that take advantage of, or mimic, this cardioprotective effect, in particular in the critical 1–3 hour window following coronary arterial occlusion.[129] An important step in the search for clinically useful cardioprotection strategies has been the recent agreement on standardized animal models for preclinical screening.[130] In addition to classic preconditioning, cardioprotection can also be induced by temporary hypoxia at body locations other than the heart (for example, a limb) prior to MI (remote preconditioning),[131] acutely following MI (post-conditioning),[132] or by subjecting the heart to mechanical stretch.[133] All these may contribute to the beneficial cardiovascular effects of regular exercise.

Novel approaches to pharmacologically induce cardioprotection include targeting signalling through mitochondrial-associated protein kinase C and its substrates, such as aldehyde dehydrogenase 2, which reduces ischemic damage by reactive aldehydesCompounds inhibiting the mitochondrial permeability transition pore (mPTP) may also mitigate changes that initiate mitochondrial-driven cardiomyocyte death.[129, 135] This being said, the mPTP blocker cyclosporine A recently failed to meet endpoints in the CIRCUS trial – a study designed to determine whether this inhibitor protected hearts from ischaemia/reperfusion injury.[136] Other experimental and clinical-stage therapeutics that may mimic pathways activated in ischemic preconditioning include phosphodiesterase 5 inhibitors (such as Viagra),[137] chloride channel and transporter modulators,[138] hydrogen sulfide,[139] rapamycin,[140] and nitric oxide (NO) donors.[141] Overexpression of extracellular superoxide dismutase has been shown to increase NO bioavailability and provide cardioprotective benefit in transgenic mouse models.[142]

Connexin hemichannels (connexons) are single membrane channels in transit to docking within gap junctions. Hemichannels have recently come into focus as another novel determinant of acute injury severity following MI.[143, 144] The majority of hemichannels in the cardiomyocyte cell membrane reside in a zone surrounding the gap junction, called the perinexus.[145] In response to an ischaemic insult, hemichannels activate, opening non-selective pores that discharge high concentrations of pro-inflammatory, pro-fibrotic molecules such as adenosine triphosphate (ATP), while admitting cytotoxic levels of Na^{2+} and Ca^{2+} into cells.[144] There is also evidence that Cx43 hemichannels act in concordance with mPTP channels in response to ischaemic injury.[146] The short peptides Gap26/27, αCT1 and Gap19 mimic key functional domains of Cx43, and have all been shown to inhibit hemichannel activity (Gap26/27 blocks gap junction channels as well).[147] Gap19 has cardioprotective effects, significantly decreasing infarct size in a mouse MI model.[144] Gap26 confers cardioprotection post-injury in a rat MI model *in vivo:* intra-arterial injection of the

peptide 30 minutes after coronary artery ligation reduced infarct size by 61%.[143] Interestingly, the same experimental hemichannel blockers have proved effective in reducing glial scarring, providing neuroprotection following ischaemic stroke in animal models.[148]

Cell therapies—Mesenchymal stromal cells (MSCs), haematopoietic and endothelial progenitor cells, as well as mononuclear cells that are derived from bone marrow and other sources are at the core of a concerted effort in experimental reparative therapy development.[149, 150] Supported by preclinical studies in animal models, several groups have tested cell therapies in patients with MI. Many, but not all, trials reported modest increases in ejection fraction or other signs of functional improvement, and some meta-analyses suggest that cell therapy with bone marrow mononuclear cells or mesenchymal cells may assist in the recovery of function of injured or diseased hearts.[149, 151]

However, thus far analyses have been based on small or moderately sized clinical investigations. Given the heterogeneity of both the cell preparations used and the patient populations studied, such analyses should be interpreted with caution.[152] The US clinicaltrials.gov website lists more than 300 trials involving cardiac cell therapy, the majority of which are early Phase I and II safety and efficacy studies. Outcomes from Phase III clinical trials involving thousands of patients are still missing but are necessary to establish whether or not largely bone marrow or adipose tissue-derived mesenchymal populations reliably provide clinically meaningful improvements, on top of current standard-of-care. A further note of caution on these therapies came from meta-analyses, such the ACCRUE review of 12 randomized clinical trials, of the effects of intracoronary cell therapy on ischaemic heart disease, which failed to identify patient benefit, in terms of either clinical events or improved left ventricular function.[153]

Cardiac stem cells have been suggested to be, or at least include, resident progenitor cell populations in the heart, with potential roles in regenerating myocardium.[154] Various surface antigens such as tyrosine-protein kinase Kit (c-Kit) or Stem cell antigen-1 (Sca-1), or spheroid cultivation assays, have been used to isolate cardiac stem cells. Engraftment of different cell populations improves cardiac function and reduces infarct area in mouse models, and two first-in-human clinical trials assessing c-Kit+ (SCIPIO)[155] and cardiosphere-derived cells (CADUCEUS)[156] reported promising results. However, the contribution of cardiac stem cells to regeneration of the heart is still a matter of debate, as is the potential for heart muscle cells to contribute to self-repair by proliferation.[157] Whereas mouse heart failure models have suggested that resident c-Kit+ cells may affect cardiac repair by direct cardiomyogenic contributions,[158] Cre-reporter-based experiments have demonstrated that c-Kit expression is largely confined to non-myocyte cardiac lineages.[159] The latter finding is consistent with other reports, which found that the cardiogenic potential of c-Kit expressing cells in the adult heart is limited,[160] and that c-Kit predominantly labelled cardiac endothelial cells in developing and adult hearts.[161] Another study suggested that the large majority of c-Kit-derived muscle cells were pre-existing c-Kit-expressing cardiomyocytes, rather than cells formed *de novo* from CSCs.[162] Similarly, lineage tracing of Sca-1-positive stromal cells revealed only a modest contribution of these cells to cardiovascular lineages after injury and during aging.[163]

Unfortunately, the debate on cardiac cell therapy has, in a few high-profile cases, moved beyond scientific controversy.[164] The emphasis of the field has also shifted from regeneration to the potential cardioprotective effects of cell therapies – effects that, in large part, are now thought to be mediated by paracrine mechanisms. This latter view is consistent with the fact that many of the paracrine factors released by the applied cells are known to influence fibrosis, angiogenesis and inflammation, and by these means, affect cardiac healing responses and scar properties.[165] Matrix remodelling can be influenced by the release of matrix-modulating enzymes, such as matrix metalloproteases,[166] or by modulating expression of tenascins.[167] The secretion of HGF,[168] adrenomedullin,[169] and thymosin-β4[170] may also directly affect cardiac fibrosis. Interleukins may act to inhibit cardiac fibroblast proliferation and collagen production as well as myofibroblast differentiation,[171] while MSC-derived leptin was shown to inhibit fibroblast activation by blocking pathways involving TGFβ/'small mothers against decapentaplegic' (Smad) and Myocardin-Related Transcription Factor-A.[172]

Detailed analysis of bone marrow mononuclear cell secretomes has led to the identification of novel cardioprotective proteins, such as myeloid-derived growth factor.[173] In addition to secreted proteins and growth factors, cells of different origins can release extracellular vesicles, such as exosomes, which contain various bioactive molecules, including microRNA.[174] Cell therapies, thus, may profoundly alter the paracrine microenvironment in the healing heart, thereby not only affecting mesenchymal cell activity and trans-differentiation, but also enhancing regeneration, potentially by inducing cardiomyocyte proliferation,[175] albeit at rates as low as 1% in 25 years.[157]

The low homing and survival rates of externally applied cells in scar tissue, however, are likely to limit the duration of paracrine protective actions.[176] Augmenting homing and survival, either by pre-treating the engrafted cells with pro-survival factors, genes or miRNA, or by preconditioning the target tissue, may enhance the efficacy of cell therapy.[176] With ongoing development of the field, it will be interesting to see whether cell-free cocktails of bioactive molecules can be formulated that supersede cell-injection based therapies.[177]

Antifibrotic therapies—Congestive heart failure, a progressive and ultimately incurable disease, is marked by the increasing inability of the ventricle to pump blood sufficiently to meet the demands of the body. Replacement of cardiac muscle by fibrotic tissue is thought to be at the pathogenic nidus of heart failure.[1, 3–6] The acute loss of muscle, such as sparked by MI, viral infection, or drug toxicity, could be the precipitating cause of heart failure, and is followed by disease progression. Fibrosis at interstitial and perivascular locations in the failing ventricle can also occur as part of longer term processes associated with aging or diseases such as hypertension, aortic stenosis, metabolic disease and obesity. In the atria, connective tissue accumulation is an underlying cause of atrial fibrillation. Preventing, or at least inhibiting, the accumulation of pathologic levels of fibrotic tissue in both ventricular and atrial myocardium is thus a major therapeutic goal.

Exploration in this area is focused on anti-fibrotic activities of established drugs, and on novel compounds. Examples of the former category include statins, which are anything but

new and are among the most widely prescribed drugs of the last quarter century. Their primary mode-of-action is to lower cholesterol levels via inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase.[178] It has emerged from preclinical and clinical studies that certain statins also inhibit scarring, fibrosis and adverse myocardial remodelling.[31, 179, 180] The mechanism through which statins inhibit fibrosis is not entirely clear, though simvastatin inhibits trans-differentiation of cardiac fibroblasts into myofibroblasts, an effect that is overcome by application of TGFβ.[181] Other established drugs that have anti-fibrotic actions include aldosterone antagonists and angiotensin receptor and ACE inhibitors.[3]

A number of recent reviews have summarized preclinical and clinical progress on novel therapeutics, which are only briefly covered here (Table 1).[1, 3–6] Experimental or clinical stage antifibrotic compounds include TGFβ pathway inhibitors,[209–214] matrix metalloproteinase inhibitors,[10] inhibitors of mast cell function,[195, 215] monoclonal antibodies neutralizing the monocyte chemo-attractant MCP-1,[188] chemokine interferon-gamma-inducible protein IP-10/CXCL10,[216] endothelin pathway inhibitors,[217] Ang-II pathway inhibitors,[184, 185] and modulators of Wnt-signalling.[196, 197]

Other new anti-fibrotic targets include the proto-oncogene ski, the basic helix-loop-helix transcription factor scleraxis, and the cell membrane proteins β3 integrin and caveolin-1. Ski inhibits TGFβ signalling by binding to downstream transcriptional regulatory proteins in the TGFβ/Smad pathway.[218] Overexpression of ski in isolated cardiac myofibroblasts was found to result in phenotypic reversion, marked by reduced collagen expression and myofibroblast contractility.[219] Scleraxis is a key regulator of collagen synthesis in cardiac fibroblasts, with potential regulatory roles in fibroblast to myofibroblast differentiation.[220]

Mice deficient for β3 integrin showed substantial decreases in pressure overload-induced fibrosis, suggesting the involvement of this integrin in maladaptive ECM accumulation.[221] By contrast, loss of caveolin-1 function in mice following MI[222] and in isolated human atrial fibroblasts[223] resulted in TGFβ1 associated fibrosis and upregulation of pro-fibrotic genes. Whilst the path to clinical translation of ski, scleraxis, and β3 Integrin to druggable targets is still in exploratory phases,[224, 225] peptidergic drugs targeting caveolin-1 function have demonstrated promise as anti-fibrotic therapeutics in studies of lung fibroblasts from scleroderma patients.[226]

Relaxin-2, a neurohormone historically linked to pregnancy and parturition, is another novel biologic compound that has made the translational jump from basic research to the clinic (under the generic name *serelaxin*), and had efficacy in clinical testing of patients with acute heart failure.[227, 228] The large placebo-controlled Phase III trial of 1,161 patients with heart failure, RELAX-AHF, reported significant improvements in patient symptoms, as well as decreases in their length of hospital stays and increased survival rates.[191] It is presently unclear what molecular mechanism underlies the effect of relaxin on fibrosis. However, experiments in cell culture models indicate that relaxin inhibits and/or reverses myofibroblast differentiation induced by angiotensin and TGFβ, as assessed by changes in gene expression and markers of cellular electrophysiology.[229, 230]

A number of miRNA-based strategies have also shown potential recently for inhibiting cardiac fibrotic processes.[231, 232] The first matrix-regulating miRNA to be identified in the heart, miR-29, targets several matrix proteins, including collagens.[40] Over-expression of miR-29 in cardiac fibroblasts reduces collagen expression, albeit modestly.[40] Antagomir-mediated silencing of miR-21 *in vivo* inhibits interstitial fibrosis and attenuates dysfunction in a murine ventricular pressure-overload model.[204] Similarly, knockdown of miR-21 by anti-sense RNA in the atria was found to suppress atrial fibrosis in a rat model of MI-induced heart failure.[233] However, in other studies, locked-nucleic acid anti-miR-based inhibition or genetic deletion of miR-21 failed to block the adverse ventricular remodelling response of mouse hearts subjected to pressure overload.[234] Differences in the anti-fibrotic response may in part rely on the chemistry that was used to inhibit miR-21.[233] Although the anti-fibrotic activity of anti-miR-21 in the heart is debated, a modified miR-21 antisense (RG-012) is currently being clinically tested in patients with kidney fibrosis (the ATHENA trial).[235] Many additional miRNAs control myocyte survival after MI, thereby indirectly or directly affecting fibrosis.[236] Inhibition of age-induced miR-34 expression can also decrease age-related cardiac fibrosis.[237] Interestingly, miRNA may not only directly or indirectly target fibroblasts, but it is also released by cardiac fibroblasts. For example, fibroblast-derived exosomal miR-21-3p (miR-21*) acts as a potent paracrine-acting RNA molecule that induces cardiomyocyte hypertrophy.[238]

All in all, present conceptual approaches for therapeutic interventions are focused on avoiding or inhibiting fibrosis and/or on turning non-myocytes into working cardiac muscle. An alternative idea, providing a lower-hanging fruit of potentially high value, will be discussed next: the making of better scars.[2]

Re-engineering scars

Utilizing the natural reparative processes of fibroblasts to modify properties of the forming cardiac scar is quietly emerging as an exciting therapeutic avenue. Part of the rationale here comes from the recognition that, whilst fibrotic responses are common in the repair processes in the human heart, not all fibroblast-mediated cardiac remodelling is detrimental.[41, 53, 94]

Fibroblast lineage studies already hint at differences in regulatory interactions that determine fibrosis in response to ischaemic versus hypertrophic diseases.[97, 99, 100] Furthermore, fibroblasts in the atrium and ventricle show differences in gene expression,[239] electrophysiological characteristics,[88, 240] and propensity for pro-fibrotic activity.[241, 242]

Cardiac scar formation is not always the result of disease. Interventions such as catheter ablation therapy intentionally generate scars in the heart to obliterate reentrant activation pathways, for example to alleviate atrial rhythm disturbances.[243] On the other hand, pediatric cardiologists face a growing (and in some ways welcome) problem of managing adult patients with morbidities that are linked to the accumulation of scar tissue resulting from childhood surgeries to correct heart birth defects.[244]

It is important in this context to recall that not all cardiac scars are created equal. Post-surgery, de-novo generation of both ECM and cellular content are involved in scar generation, while post-ablation scars are re-cellularized on the background of pre-existing ECM. Post-MI scars, in contrast, contain not only native ECM but also surviving heart cells of different types, albeit with reduced myocyte content, caused by the pronounced sensitivity of these cells to oxygen starvation. These pose three potentially quite different pathophysiological settings.

The ability to personalize the properties of cardiac scar tissue to meet the needs of different types of scar and patient is thus an interesting and currently unmet clinical need.[2, 245]

Therapeutic potential of modifying scar mechanics

Notable aspects of the effort to “engineer a better scar” include insights into biomechanical inputs in fibrotic tissue differentiation, as well as the role of scar structure in determining the efficiency of cardiac output. Ventricular function has recently been recognized to be affected more by the alignment of collagen fibers within post-MI scars than by scar stiffness or collagen density.[246] Collagen alignment, in turn, is a function of mechanical stress patterns that cardiac scars are subjected to during the contraction cycle.

Scar mechanical properties are also critical factors for the genesis of complications arising from ischaemic heart disease — including infarct rupture and scar expansion — that can occur after MI. Experimental therapies that attempt to modify mechanics and inhibit pathological remodelling of the infarct include mechanical restraints that limit or direct the motion of scars during the heartbeat,[247, 248] injection of polymers to modify scar rigidity,[249–251] MMP9 loss-of-function,[252] and hydrogels containing tissue MMP inhibitors or viruses over-expressing them.[253, 254] It is also of note that cardiac resynchronization therapy, an approved treatment for dilated heart failure, can promote reverse remodelling by normalizing regional stress/strain patterns across the infarcted ventricle.[255]

The risk of post-MI scar dilation and rupture is an important safety consideration for therapies targeting scar mechanical properties. The ventricle of the adult human heart generates systolic pressures of 120 to 180 mmHg, similar to mice and rats post-partum, but nearly one order of magnitude higher than pressures in utero (~30 mmHg) and about two orders above that of animals in whom myocardial regeneration is maintained into adulthood, such as the zebrafish (~2 mmHg).[256] It seems a reasonable conjecture that the evolutionary emergence of higher blood pressure in adult mammals may have had an impact on the risks and benefits of healing the heart by scarring versus regeneration. In an illustration of this potential trade-off, adult mice in whom the gene encoding the ECM protein periostin has been knocked out are prone to ventricular rupture following MI.[81, 257] However, those periostin knockout mice that survived exhibited reduced levels of scarring and improved cardiac performance.[81]

One might expect that therapies based on cardiomyogenic stem cells also pose increased risk of rupture and ventricular dilation, wherein infarcts could be more likely to show mechanical failure when healing is shifted towards regeneration and away from expeditious formation of a mechanically strong scar. Bone marrow-derived cells, which have limited cardiomyogenic

potential, may be beneficial in this respect. Pharmacologically induced increases in recruitment of bone marrow-derived mesenchymal progenitor cells to MI in a mouse model was reported to be associated with reduced rates of ventricular rupture and death.[258] These effects may provide a further explanation for how bone marrow-derived cell infusion contributes to clinical improvements following MI, and also suggest novel strategies to manipulate these cells, which to home-in on infarcts, to generate better scars. In an example of this approach, injection into femur bone marrow of a lentivirus expressing a small hairpin RNA targeting periostin prior to ventricular injury was reported to reduce scar size and improve cardiac function – without ventricular rupture that was found to occur following MI in mice with a global knockout of periostin.[259] In this experiment, periostin knockdown was limited to bone marrow cells migrating to the MI, suggesting that selectively decreasing periostin expression in this extracardiac cell population may provide a therapeutic path to reduce scarring, and possibly promoting regenerative healing, following cardiac injury.

Therapeutic potential of modifying scar electrical properties

Fibrous, collagen-rich tissues in the heart have traditionally been viewed solely as non-conducting and electrically insulating structures. Undoubtedly, that is one of the most relevant functional effects of fibrosis, as observed more than a century ago.[260] However, newer data has led to a more nuanced understanding of the electrical properties of cardiac scars and the implications of the electrical interaction between myocytes and non-myocytes.[7, 21, 261] Since the 1960s, it has been known that fibroblasts and myocytes can couple electrically *in vitro*,[23] and more recently it was confirmed that cultured fibroblasts can bridge the transmission of action potentials (APs) between otherwise separated clusters of myocytes across gaps of up to 300 μm.[34] In an analogous example from the clinical literature, 10–20% of heart transplant recipients have been reported to develop electrical coupling across scar tissue at suture lines separating donor and recipient heart tissue.[262] Similarly, electrical conduction across fully transmural scars can be seen after surgical correction of cardiac birth defects.[263]

Myocyte-fibroblast coupling may occur via connexin-based gap junctions,[20, 264–266] which are far from uncommon in native myocardium.[267] However, functionality of connexin-based fibroblast-myocyte coupling in native cardiac tissue has been corroborated only in rabbit sino-atrial node tissue.[19] Underlying structures may also involve filamentous projections made by mesenchymal cells, called tunnelling nanotubes, through which ions and small molecules can be directly exchanged.[268] Ephaptic conduction, a non-gap junction coupling-based method to transfer currents between cells, has been reported between myocytes and could also have a role in myocyte-fibroblast coupling.[269, 270]

In situ, cardiac fibroblasts exhibit complex and extended flat shapes, with irregular folds and elongated cell processes.[18] To give a sense of their extended spatial scale *in vivo*, cardiac fibroblasts are typically an order of magnitude larger in the intact heart than when freshly isolated.[2] Fibroblasts have membrane potentials of between −10 and −50 mV and a relatively high membrane resistance.[2] Their extended morphology and elevated membrane resistance enhances the ability of fibroblasts to act as passive, long-distance conductors of electrical signals *in vivo*, including APs, when coupled to electrically active cells such as

myocytes.[264, 271] In quantitative mathematical simulations of heterocellular electrical cross-talk in the 1990s, using 'passive' fibroblast models, fibroblasts that were electrically coupled to muscle cells could affect cardiomyocyte excitability, altering heart rate and rhythm.[272] More recently, based on emerging experimental insight into their electrophysiology, detailed ionic models of cardiac fibroblasts have been developed.[273] Modelling studies have since provided indications that activated fibroblasts (myofibroblasts) may have specific electrophysiological relevance for arrhythmogenesis,[274] a phenomenon that may relate to fibroblast-myocyte coupling and include changes in fibroblast membrane ion currents that occur following MI.[2, 5, 7, 274–277] Both passive and active fibroblast models have been productive for theoretical assessment and hypothesis testing, at times preceding experimental validation.[278–281] A major step towards more realistic computer simulations occurred with the advent of detailed three-dimensional scar reconstructions,[282] paving the way towards clinically relevant prediction of scar effects on heart function, though thus far these studies have focussed largely on the classic view of scars as electrical insulators.[283–285]

In an influential study, ventricular scars were shown to be capable of conducting electrical signals;[38] optically mapped AP waveforms were observed to conduct into scar tissue in a rabbit transmural MI model. Infarct tissue continued to support conduction even after sub-endocardial muscle layers were chemically ablated, thus excluding the possibility of electrical contributions from surviving contiguous myocardium. This observation was followed by reports from other teams,[286, 287] indicating the possibility that nonmyocytes may serve as conduction pathways that link surviving myocyte islands inside post-MI scar tissue, thus allowing trans-scar conduction. Further support for this theory was provided at a recent Keystone conference, where data were presented demonstrating Cx43-dependent conduction into scar tissue in a fully transmural ventricular injury in a transgenic mouse model;[288] another group reported direct evidence of conduction into non-myocytes of the scar border zone, using a fluorescent voltage reporter genetically targeted to non-myocytes by the WT1 promoter.[289]

Non-myocyte based trans-scar conduction could be used in novel therapeutic approaches. If, in a clinical setting, scar tissue could be rendered electrically "invisible" with respect to the propagating impulse, then the potential for infarcts to cause life-threatening arrhythmias might be reduced. For example, engraftment of Cx43-expressing cells into murine scar tissue prevents post-MI arrhythmogenesis.[39] A peptide mimetic of the Cx43 carboxyl-terminus (αCT1) has been reported to reduce hemichannel activity and increase intercellular coupling by enhancing the recruitment of undocked connexin hemichannels into gap junctions.[145] Similar to the engraftment of Cx43-expressing cells post-infarction,[39] treatment of mouse ventricular injuries with αCT1 reduced the incidence of arrhythmia and increased conduction velocity, including through the infarcted section of the ventricle.[198]

Tissue-engineered constructs containing cardiac myocytes and fibroblasts have also been used successfully to conduct excitation from atria to ventricles in cases of experimental atrio-ventricular conduction block.[290] This is another potential therapeutic application that depends on efficient heterocellular coupling, both among tissue graft components and at the

graft-myocardial interface, both of which involve propagation of electrical signals across non-myocytes.[261]

The goal of engineering more conductive scars in post-MI patients is contrasted by the need for certain types of scars to remain impenetrable to electrical inputs from surrounding myocardial tissues, such as in the context of atrial ablation.[243] For this procedure, focal energy delivery, e.g. from a radiofrequency catheter, is used to generate lesions that interrupt conduction of spurious excitation waves, thereby terminating atrial arrhythmias. Unfortunately, a large proportion of patients show re-emergence of functional conduction across intra-procedurally established ablation lines.[291] Extrapolating from (and 'inverting') the electrophysiological effects of Cx43 over-expression in post-MI scar tissue, a decrease in Cx expression in atrial fibroblasts could potentially provide an approach to improving outcomes of atrial ablation procedures. Alternatively, one could build on an engineering approach in which Cx43-expressing fibroblasts were first transfected to express voltage-sensitive potassium channels, such as Kv1.3, and then engrafted into rat hearts.[292] These grafts of clustered mesenchymal cells were found to alter local electrophysiological properties and, owing to their enhanced potassium current, reduced automaticity and prolonged refractoriness of surrounding electrically coupled cardiac myocytes. This concept, pioneered in ventricular tissue, could be adapted to counter trans-scar conduction across atrial ablation lines.

Outlook

Research over the past 25 years has identified the cardiac fibroblast as a cell with an unexpected range of effects, integrating and organizing myocardial structure and function at various levels, from local signalling to global electro-mechanical function. There has been further insight into the well-established roles of fibroblasts in cardiac structure and ECM generation, together with an expanding appreciation of their paracrine effects, functions as mechanical and electrical signalling hubs, and roles in myocardial repair. There can be little doubt that there will be new surprising roles for fibroblasts in homeostasis and disease, but this is a suitable moment to take stock of this protean cell. By analogy to the glia of the central nervous system, which was once regarded as a trophic glue but is now recognized as a structure whose relevance for tissue function is on par with that of the neural network itself, we know too little about the relevance of non-excitable cells in homeostasis of excitable tissues and organs. What we do know is that the replacement of excitable cells, whether by gliosis or fibrosis, is a chief element of pathogenic processes in diseases of the brain and heart.

It is time, therefore, to shift our appreciation of cardiac mesenchymal cells away from viewing them as a passive component in an integrated and dynamic tissue system – diverse in ontogeny and inseparable from the myocardial syncytium but otherwise secondary in relevance – to one where they are credited with active roles in complex, multifunctional regulatory processes crucial not only for development but also for structural maintenance and functional operation of the heart.

The concept that fibroblasts link together to form a cardiac sub-system of co-equal importance to the syncytial network of myocytes should inform our approach to targeting or utilizing mesenchymal cells in therapies for heart disease. In this respect there are a number of exciting opportunities and important questions. The implications of complex sets of intercellular interactions, both homocellular and heterocellular, are poorly understood, and modulating cell-to-cell communication in the heart via biochemical and biophysical factors may provide new therapeutic targets. Novel paracrine factors could be identified by analyzing the secretome of cells – as has been done for cells used for cell therapy.[173] Recent studies suggest that among the many long non-coding RNA molecules, some may code for small peptides with potent biological activity.[293] Similarly, well-known cardiac proteins such as Cx43 can generate alternative bioactive peptides in response to ischaemia by cap-independent translation,[294, 295] peptidase processing,[296] or as a docking motif for exosome-cell communication,[297] further suggesting the possibility of new regulatory molecules and mechanisms that have been overlooked so far. In addition to proteins and peptides,[298] metabolites (e.g. lipids, metabolic intermediates etc.) may be identified by new “omics” tools as structural and functional regulators of the heterocellular heart. Development of pharmaceutically tractable compositions and delivery systems, including cell-specific targeting, are among the hurdles that must be overcome if these newly identified factors are to be harnessed as therapies for improving cardiac health.

Better markers and lineage tracing tools are needed for cardiac mesenchymal cells. Such tools would enable the exploration of the regulation of cell plasticity, allowing insight into the heterogeneous origins of fibroblasts, processes such as EMT, EndoMT and (reverse) mesenchymal-endothelial transition, and their various response patterns to physiological and pathophysiological challenges. Improved lineage tools may also provide us with the knowledge necessary for co-opting fibroblast behaviours, such as homing to injured loci, as a treatment modality. One might envisage genetic modifications of these migratory cells to carry payloads to damaged myocardial tissues in personalized medicine-type approaches. Further, the ability to more reliably trace cell fate would enable a more thorough understanding of mechanisms underlying fibroblast reprogramming and cell therapies, thus moving us closer to being able to comprehensively repair and/or regenerate the damaged heart.

In the meantime, the more modest goal of modifying scar tissue for patient benefit seems to be a useful tangible objective. Humans and other mammals heal damaged hearts by scarring and fibrosis. We should take a cue from Mother Nature, and determine how to therapeutically nudge or re-engineer this process, to improve clinical outcomes for patients with heart disease.

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Box 1

Effects of fibroblasts on cardiac physiology

Fibroblasts affect numerous aspects of cardiac form and function.

Altered histoanatomy:

- adaptive fibrosis
- remodelling of the extracellular matrix (ECM)
- increased tissue stiffness
- contribute to heart failure

Changes in biophysical signalling:

- alterations in connexin localisation and function
- altered conduction into the scar
- increased ECM can act as an electrical insulator, thereby reducing electrical connectivity

Changes in biochemical signalling:

- adaptive hypertrophy
- increased inflammation
- apoptosis, resulting in muscle loss

Cell differentiation:

- Fibroblast to myocyte transdifferentiation
- Endothelial- or epithelial-mesenchymal transition
- Fibroblasts may affect the microenvironment by paracrine signaling

These fibroblast-induced changes could be harnessed to regenerate or repair muscle. For example, fibroblasts could be targeted in patients with atrial or ventricular fibrillation to prevent the disease or improve the efficacy of catheter ablation, or to make scars in patients who have had myocardial infarctions electrically invisible.

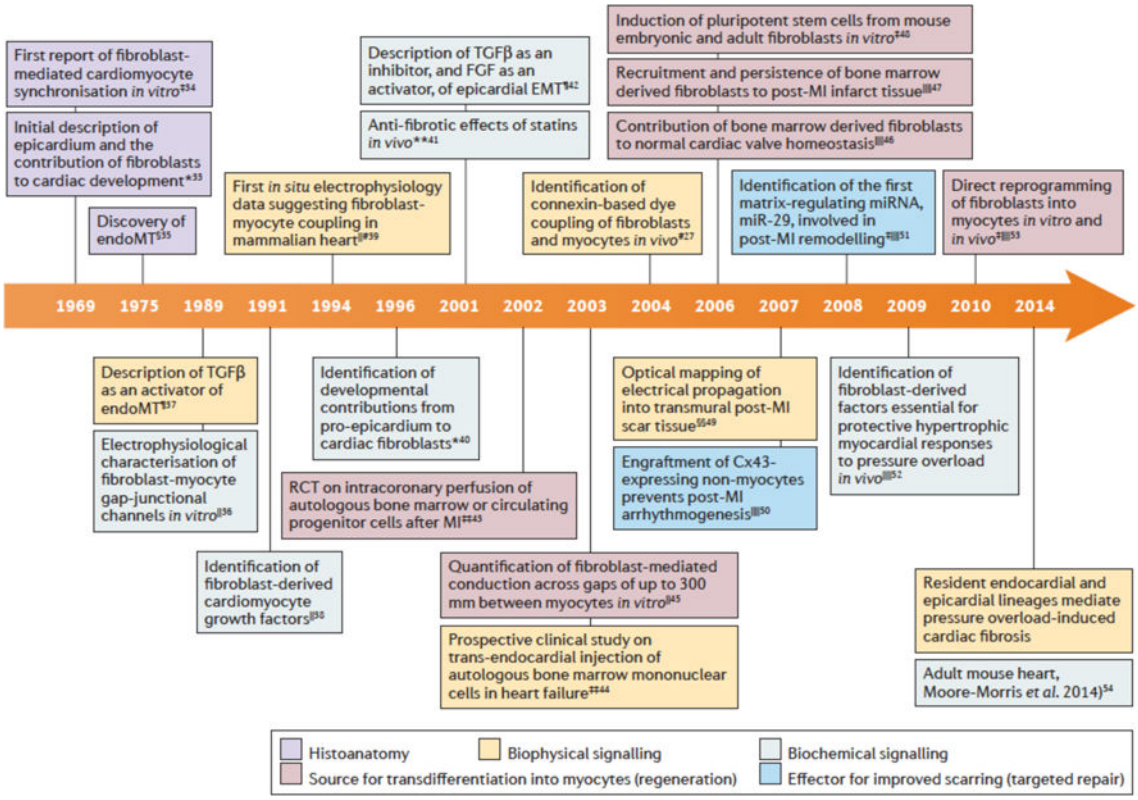

Figure 1. Key developments and changing focus of cardiac fibroblast research

This timeline shows key articles on cardiac fibroblasts. Early research in this area was predominantly descriptive of histoanatomical structures, then changed to also describing biophysical and biochemical signalling. Since the early 2000s cardiac fibroblast research has also included the roles of fibroblasts in myocyte transdifferentiation as a potential source for regeneration as well as their potential to be utilized to improve scarring. *Embryonic chick heart; ‡cultured cells from embryonic mouse heart, §embryonic rat heart, ‖ cultured cells from neonatal rat heart, ¶cultured cells from embryonic chick heart, #adult rat heart, **transgenic rabbit model of hypertrophic cardiomyopathy, ‡‡humans, §§adult rabbit heart, ‖ ‖ adult mouse heart. Cx43, connexin 43; EndoMT, endothelial to mesenchymal transition; FGF, fibroblastgrowth factor; MI, myocardial infarction; miRNA, microRNA; RCT, randomized clinical trial; TGFβ, transforming growth factor β.

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k) Assmus et al., *Circulation*: 2002, 106, 3009–17[32]

l) Perin et al., *Circulation*: 2003, 107, 2294–302[33]
m) Gaudesius et al. *Circ Res* 2003, 93:421–8[34]
n) Camelliti et al. *Circ Res* 2004, 94:828–835[19]
o) Visconti et al., *Circ Res* 2006, 98:690–6[35]
p) Möllman et al., *Cardiovasc Res* 2006, 71:661–71[36]
q) Takahashi and Yamanaka, *Cell*, 126, 2006 663–76[37]
r) Walker et al. *J Cardiovasc Electrophysiol*. 2007 Aug;18(8):862–8[38]
s) Roell W. et al. *Nature* 2007, 450, 819–824[39],
t) van Rooij et al., *Proc Natl Acad Sci U S A*, 2007 105, 13027–32[40]
u) Takeda et al. *J Clin Invest*, 2009, 120, 254–65[41]
v) Ieda et al, *Cell* 2010/142: 375–386[42]
w) Moore-Morris et al. *J Clin Invest*. 2014 Jul;124(7):2921–34[43]

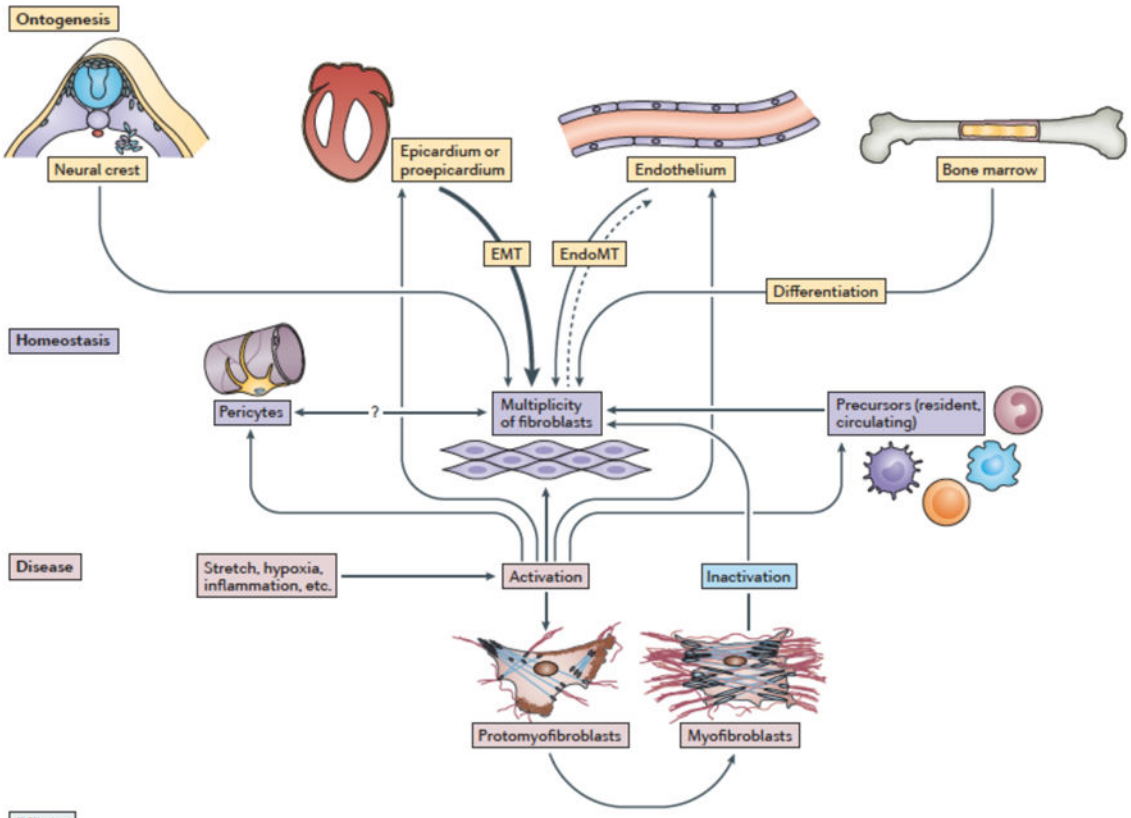

Figure 2. Cardiac fibroblast and myofibroblast origins in health and disease

Cardiac fibroblasts originate from various sources, including the (pro-)epicardium, cardiac endothelium, neural creast and bone marrow. They form amultiplicity of cells, with hitherto ill-defined precursurs (both resident and circulating), subtypes, and relations to pericytes. Fibroblast activation by pathological stimuli, including stretch, hypoxia, and inflammation, lead to phenotype-conversion via proto-myofibrobalsts to myofibroblasts. For detailed explanations, see text.

Table 1

Selected compounds and interventions that may reduce or reverse fibrosis*

Drug class or intervention	Target	Anti-fibrotic biological mode-of-action	Potential indication
Drugs and biologic compounds			
ACE inhibitors (e.g. enalapril)	Angiotensin converting enzyme	Inhibit fibroblast activation, macrophage attracting enzymes, ECM deposition	Hypertension, ischaemic heart disease, heart failure[182]
Aldosterone inhibitors (e.g. spirolactone)	Mineralocorticoid receptors	Reduce oedema, cardiac load	Heart failure[183]
Statins (e.g. simvastatin)	Co enzyme A-reductase	Reduce lipid levels, inhibit fibroblast activation	Ischaemic heart disease, heart failure, atrial fibrosis[179, 181]
Angiotensin receptorinhibitors (e.g., losartan)	AT1 receptor	Inhibit fibroblast activation, ECM deposition	Heart failure[184, 185]
Endothelin receptor inhibitors (e.g. bosentan)	ET receptors	Reduce fibroblast ECM deposition	Failed Phase III testing for heart failure and ischaemia reperfusion injury {Rodriguez-Pascual, 2014 #14279; Singh, 2006 #14280}
Beta blockers (e.g. propranolol)	Beta adrenergic receptors	Inhibit fibroblast activation, ECM deposition	Heart failure, reverse remodelling[186]
Acetylsalicylic acid (e.g. asprin)	Cyclooxygenase, redox-sensitive transcription factors	Inhibit fibroblast activation	Cardiac hypertrophy, reverse remodelling[187]
MCP-1 neutralizers (e.g., anti-CCL2 antibody)	Monocyte Chemo-attractant Protein-1	Inhibits macrophage and fibroblast proliferation, and induction	Myocardial infarction, ischaemia reperfusion injury[188]
PPARγ agonists (e.g. thiazolidinediones)	Nuclear hormone receptor peroxisome proliferator activated receptor-gamma	Inhibit fibroblast activation, ECM deposition	Hypertrophic cardiomyopathy, heart failure, myocardial infarction[189]
MMP inhibitors (e.g., batimas)	Matrix metalloprotein	Inhibit extracellular matrix remodelling	Cardiac scar re-engineering. Failed Phase III testing for heart failure[190]
Relaxin inhibitors (e.g., serelaxin)	Relaxin-2	Inhibit fibroblast activation ECM expression	Heart failure, atrial fibrosis[191]
PDGFR inhibitors and anti-PDGFR antibodies	Platelet derived growth factor receptor-α	Inhibit atrial fibrosis	Atrial fibrosis/fibrillation[192]
Mast cell inhibitors (e.g., nedocromil)	Mast cells	Inhibit mast cell activation and histamine release	Heart failure, hypertension, ischaemia reperfusion injury[193]
Hyaluronan inhibitors (e.g., exogenous HA)	Endogenous hyaluronan organization	Inhibit fibroblast activation	Myocardial infarction[194]
Chymase inhibitors (e.g., TY51469)	Chymase	Attenuation of inflammatory pathways, inhibition of fibroblast proliferation	Ischaemia reperfusion injury[195]
Wnt inhibitors (e.g. sFrpl)	Canonical Wnt signalling	Inhibit pro-fibrotic cytokine signalling, ECM remodeling	Myocardial infarction[196, 197]
Connexin inhibitors (e.g., αCT1)	Connexin 43	Inhibit connexin hemichannel activity	Myocardial infarction, atrial fibrosis[143, 144, 198]
Cardioprotective drugs (e.g., mitochondrial PTP Inhibitors)	Mitochondrial permeability transition pore in myocytes	Prevention of muscle loss and replacement by scar during ischaemia	Myocardial infarction. {Duncker, 2000 #14300; Chen, 2008 #7585} CIRCUS trial failed to meet primary endpoints[136]
Cell and tissue interventions			
Bone marrow-derived stem cells (e.g., BMCs, CD34+ cells, MSCs)	Multiple cells: endothelial cells, myocytes, fibroblasts	Paracrine modulation of wound healing and scarring	Myocardial infarction, ischaemic heart failure[149, 150, 153]

Drug class or intervention	Target	Anti-fibrotic biological mode-of-action	Potential indication
Adipose-derived cells (e.g. MSCs)	Multiple cells: endothelial cells, myocytes, fibroblasts	Paracrine modulation of wound healing and scarring	Myocardial infarction[200]
Endogenous cardiac stem cells (e.g., cardiospheres, c-Kit+ cells)	Multiple cells: endothelial cells, myocytes, fibroblasts	Paracrine modulation, Possibly through myocyte differentiation	Myocardial infarction, ischaemic heart failure[155, 156]
Cardioprotective procedures (e.g., remote preconditioning)	Myocytes	Prevention of muscle loss and replacement by scar during ischaemic events	Myocardial infarction[201]
Fibroblast reprogramming (e.g. GMT)	Cardiac fibroblasts	Convert scar-forming fibroblasts into myocytes	Myocardial infarction[110–112, 122, 202]
Cellularized ECM Scaffolds (e.g., with iPSC-derived myocytes)	Cardiac scar	Revision/replacement of scar tissue with new myocardium	Myocardial infarction[13, 15, 203]
miRNAs, lncRNAs etc			
AntimiR-21	Fibroblasts	Inhibition of fibrosis	Kidney fibrosis, myocardial infarction[204]
AntimiR-92	Endothelial cells	Improvement of angiogenesis, cardioprotection	Myocardial infarction[205]
Other			
Angioplasty	Blocked coronary arteries	Balloon catheter used to re-establish blood flow to prevent replacement of muscle by scar	Myocardial infarction[194]
Thrombolytic therapy (e.g., tPA)	Blocked coronary arteries	Clot-dissolving drug used to re-establish blood flow to prevent replacement of muscle by scar	Myocardial infarction[182, 206]
Cardiac resynchronization therapy	Ventricular stress/strain patterns	Promotes reverse remodelling	Myocardial infarction[207]
Dietary sugar reduction	p300	Inhibit fibroblast activation, ECM expression	Diabetes-induced heart failure[208]

* Substances or strategies that are or are close to clinical use are included.