Review article

Cardiac fibroblasts and mechanosensation in heart development, health and disease

Maurizio Pesce,1*; Georg N. Duda, 2,3; Giancarlo Forte, 4; Henrique Girao, 5,6; Angel Raya, 7,8,9; Pere Roca-Cusachs, 10; Joost P. G. Sluijter, 11; Carsten Tschöpe, 3,12,13; Sophie Van Linthout, 3,13*

*Corresponding authors: e-mail: maurizio.pesce@ccfm.it ; sophie.van-linthout@bih-charite.de

1. Unità di Ingegneria Tissutale Cardiovascolare, Centro Cardiologico Monzino, IRCCS, Milan, Italy.
2. Berlin Institute of Health at Charité - Universitätsmedizin, Julius Wolff Institute, Berlin, Germany.
3. Berlin Institute of Health at Charité - Universitätsmedizin, BIH Center for Regenerative Therapies, Berlin, Germany.
4. International Clinical Research Center (FNUSA-ICRC), St. Anne's University Hospital, Brno, Czech Republic.
5. Center for Innovative Biomedicine and Biotechnology, Coimbra Institute for Clinical and Biomedical Research, Faculty of Medicine, University of Coimbra, Coimbra, Portugal.
6. Clinical Academic Centre of Coimbra, Faculty of Medicine, University of Coimbra, Coimbra, Portugal.
7. Regenerative Medicine Program, Bellvitge Institute for Biomedical Research, Program for Clinical Translation of Regenerative Medicine in Catalonia, L'Hospitalet de Llobregat, Barcelona, Spain.
8. Biomedical Research Networking Center - Bioengineering, Biomaterials and Nanomedicine, Madrid, Spain.
9. Catalan Institution for Research and Advanced Studies, Barcelona, Spain.
10. Institute for Bioengineering of Catalonia, Barcelona Institute of Science and Technology (BIST), Barcelona, Spain.
11. Division Heart and Lung, Cardiology, Experimental Cardiology Laboratory, Regenerative Medicine Center, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands.
12. Charité - Universitätsmedizin, Department of Cardiology, Campus Virchow Klinikum, Berlin, Germany.
13. German Centre for Cardiovascular Research (DZHK), Berlin, Germany.

Key points

- The sensing of mechanical tissue properties is a process related to cell differentiation, maturation and pathology in multicellular organs such as the heart.
- Remodelling of the cardiac extracellular matrix, which occurs as a consequence of a pathological stimulus, induces changes in the mechanical properties of the myocardium.
- Variations in the mechanical properties of the myocardium are related to the activation of pro-fibrotic cells (so-called myofibroblasts).
- Mechanical cues can potentiate pro-fibrotic humoral signalling.
- The identification of molecular pathways involved in mechanosensation of myofibroblasts facilitates the identification of therapeutic targets that can reverse mechanically induced pathological activation.
- The possibility that interfering with mechanical cues in vivo might result in cardiac regeneration opens new therapeutic avenues in cardioprotection.

Keywords

Cardiovascular biology; Heart development; Heart failure

Abstract

The term ‘mechanosensation’ describes the capacity of cells to translate mechanical stimuli into the coordinated regulation of intracellular signals, cellular function, gene expression and epigenetic programming. This capacity is related not only to the sensitivity of the cells to tissue motion, but also to the decryption of tissue geometric arrangement and mechanical properties. The cardiac stroma, composed of fibroblasts, has been historically considered a mechanically passive component of the heart. However, the latest research suggests that the mechanical functions of these cells are an active and necessary component of the developmental biology programme of the heart that is involved in myocardial growth and homeostasis, and a crucial determinant of cardiac repair and disease. In this Review, we discuss the general concept of cell mechanosensation and force generation as potent regulators in heart development and pathology, and describe the integration of mechanical and biohumoral pathways predisposing the heart to fibrosis and failure. Next, we address the use of 3D culture systems to integrate tissue mechanics to mimic cardiac remodelling. Finally, we highlight the potential of mechanotherapeutic strategies, including pharmacological treatment and device-mediated left ventricular unloading, to reverse remodelling in the failing heart.

Introduction

Myocardial cells are continuously exposed to mechanical forces. These forces include: the stretch and compression of contractile and non-contractile cells as a result of the rhythmic beating; shear stress owing to blood flow on endothelial cells of the heart cavities and in the coronary vasculature; tension caused by local stiffness; and active contraction during tissue remodelling. In this environment, cardiac fibroblasts, which make up 10–25% of the total number of cells in the myocardium[1,2], have an essential role in maintaining structural and mechanical cardiac integrity. Although the function of cardiac fibroblasts has been under-appreciated compared with those of other cardiovascular cell types, their importance in myocardial homeostasis has been reconsidered in the context of mechanically driven tissue morphogenesis3. Indeed, cardiac fibroblasts are mechanosensitive and mechanically active cells that can translate mechanical stimuli into intracellular signals, which in turn modulate gene expression and the epigenetic landscape[3]. These features have also been associated with other mesenchymal cells[4,5,6], such as those derived from the bone marrow stroma. In addition, cardiac fibroblasts are indispensable for the organization of the cardiac extracellular matrix (ECM), the elastic network in which cardiomyocytes are embedded and against which they deploy their force to generate contraction–relaxation cycles. The structure and the biophysical features of the cardiac ECM are directly linked to the organization and the mechanical function of contractile cells. Studies have shown that the intrinsic mechanical properties of the cardiac ECM, as dictated by cardiac fibroblasts, are associated with cardiac physiology from the earliest stages of the developmental process[7,8]. The cardiac ECM has a role as a potent effector of cardiac morphogenesis, in the switch between fetal proliferative and early postnatal hypertrophic growth, and in the active intercellular crosstalk between cardiac-resident and inflammatory cells in the cardiac repair process subsequent to ischaemic and metabolic insults[7,8]. Ultimately, the maladaptive cardiac remodelling process orchestrated by

pathologically activated cardiac fibroblasts (so-called myofibroblasts[9]) is, at least in part, controlled by mechanical signalling in concert with signal transduction pathways that are activated by inflammatory mediators and pro-fibrotic factors[4,5].

Although novel insights from 2D and 3D cell cultures and live cell interferometry indicate that the topological arrangement of fibroblasts has a role in regulating their cellular plasticity and reparative capacity[10], further research is warranted to unravel the responsiveness of fibroblasts to their environment, which depends on the disease and stage of fibrogenesis. A good understanding of this process and the mechanisms involved in maintaining cardiac fibroblasts in a quiescent state under normal cardiac workload is particularly important given that mechanical-dependent differentiation of fibroblasts into myofibroblasts is inactive under healthy conditions[11].

In this Review, we describe the mechanical role of cardiac fibroblasts during cardiac development, with an emphasis on the capacity of fibroblasts to 'perceive' mechanical cues and to regulate the biophysical properties of the myocardium. Next, we discuss how the fibrotic heart is the result of the integration of biohumoral and mechanical cues by cells involved in force generation (cardiomyocytes) and cells involved in maintaining the structural integrity of the organ (cardiac fibroblasts). Finally, we highlight how the modulation of cardiac fibroblast mechanosensation and contraction affects the biohumoral regulation of cardiac fibrosis. These findings provide a basis for the development of therapeutic strategies, such as the generation of cardiac tissue constructs, to limit cardiac fibrotic growth and the progression of heart failure and, possibly, to regenerate the heart.

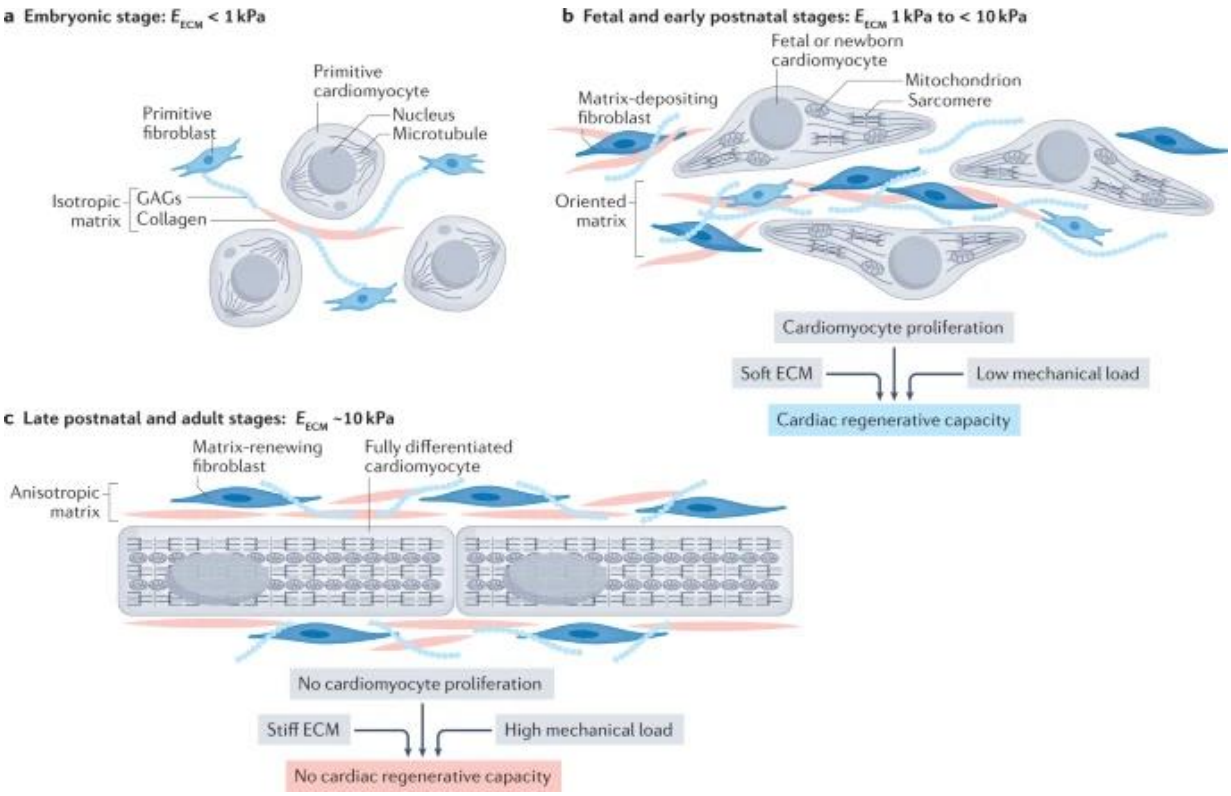
Biophysical regulation of cardiac (re)generation

Fibroblast–cardiomyocyte interaction

Unlike other organs such as the brain, where the developmental process is not associated with substantial changes in tissue biophysical characteristics[12], the heart undergoes continuous mechanical maturation from the earliest stages of morphogenesis until its final shaping[12]. This process, which occurs from the very beginning of cardiac tube formation, involves regulated mechanical crosstalk between cardiomyocyte progenitors and fibroblast progenitors[13,14,15]. Fibroblast progenitors migrate from various areas, such as the embryonic epicardium and the endocardium, to acquire a matrix-organizing function[16]. A key feature of this dynamic process is the rapid and stage-specific stiffening of the cardiac ECM. In chick embryos, for example, the stiffness of the heart increases at a rate of 0.3 kPa per day starting on embryonic day 1 (E1; Young's modulus (E) = ~0.4 kPa), and continues to increase until E14, when it reaches a level similar to that of the adult heart (E = ~10 kPa)[17]. Although the increase in stiffness is attributable to modifications in ECM composition (including an incremental increase in collagen I deposition by cardiac fibroblasts) and changes in the expression of contractile proteins (such as cardiac myosin and actinin) or junctional or adhesion molecules in the contractile cells[17], the progressive stiffening of the ECM favours mechanical maturation of the primitive cardiomyocytes to promote striation and sarcomere strengthening[12]. Interestingly, this process occurs independently of the electrical coupling of the primitive cardiomyocytes, thereby

suggesting a primary effect of ECM stiffness on primordial functional specification of the cardiac contractile cells[18]. This finding corroborates our understanding of the close relationship between the regulation of the biophysical properties of the cardiac ECM by the fibroblasts and the functional maturation of cardiomyocytes in the developing myocardium[17] (Fig. 1).

Fig. 1: The mechanical framework of myocardial cell maturation.



a, The mechanical maturation of the myocardial tissue begins shortly after formation of the cardiac embryonic primordia by interaction of the presumptive cardiomyocytes with the extracellular matrix (ECM). At this stage, the stiffness of the ECM ranges from 200 Pa to 1 kPa, which favours the activation of mechanosensitive Ca2+ channels that are thought to be involved in the mechanical maturation of the cells[9,14,15]. **b**, During fetal life and for a short time after birth, the cardiac ECM undergoes further mechanical maturation with deposition of collagen by secretory fibroblasts and strain-dependent orientation of ECM components. At this stage, ECM stiffness reaches its final compliance of 10 kPa and cardiomyocytes can still proliferate, thereby making cardiac regeneration possible. **c**, The increase in workload as a result of the shift in the environmental conditions after birth is the final event in the mechanical maturation of the heart[19]. At this stage, cardiomyocytes start growing by hypertrophy and cease to proliferate. This event makes cardiomyocyte differentiation apparently irreversible and cardiac regeneration very unlikely[19,99,251,252,253]. EECM, Young's modulus of the extracellular matrix; GAG, glycosaminoglycan.

Mammalian heart growth during fetal development is mainly sustained by an increase in cardiomyocyte numbers (hyperplasia), whereas after birth, the expansion of cellular size supports cardiac growth through hypertrophy. In many species, the shift between hyperplasia and hypertrophy in the heart is associated with an apparently irreversible cardiomyocyte withdrawal from the cell cycle and failure to complete cytokinesis[19]. However, in adults, cardiomyocytes might re-enter the cell cycle and undergo karyokinesis after the activation of a transcriptional reprogramming that is similar to the fetal proliferative transcriptional programme (for example, by upregulation of certain microRNAs[20]). The inhibition of cytokinesis by ECM stiffness-dependent sarcomere maturation might be essential in preventing uncontrolled growth of the newly formed myocardium and in reducing the risk of diseases such

as arrhythmias[21]. The increase in cardiac workload at birth, which occurs as a result of the sudden shift from the reduced gravitational forces experienced by the fetus in the womb to the normal gravitational forces experienced by the newborn, might be the ultimate cause of sarcomere maturation and the increase in cardiomyocyte size[22]. This sudden shift might hinder the formation of the mitotic spindle. In line with this hypothesis, in vertebrates such as amphibians (for example, urodeles[23]) and fish (such as zebrafish[24,25]), which require a lower degree of adaptation to the differences in gravity between the fetal and the adult stages, cardiomyocytes retain the capacity to re-enter the cell cycle and regenerate the heart after injury. Intriguingly, this event is connected to a transient softening of the ECM and a dedifferentiation of the cardiomyocyte contractile apparatus[26]. Experiments in these animal models have also clarified the role of fibroblasts in this well-coordinated process. In zebrafish, the structural and mechanical reorganization of the cardiac ECM after injury involves the fibrotic activation of cardiac fibroblasts, characterized by an increase in periostin expression (a marker of fibrillogenesis)[27]. Unlike in mammalian species, cardiac fibroblast activation after injury occurs only transiently in zebrafish, paralleled by the transient dedifferentiation of cardiomyocytes, which re-enter the cell cycle and regenerate the heart[27]. Finally, the differences between various teleost fish species endowed with different cardiac regenerative capacities have provided an insight into how the remodelling of the ECM by fibroblasts could instruct the heart regeneration process. Such insights can contribute to the development of species-specific strategies to induce cardiac regeneration. For example, in a side-by-side comparative study of medaka (a teleost without cardiac regeneration capacity) versus zebrafish, the persistent activation of cardiac fibroblasts expressing periostin in medaka at a late stage after injury (30 days after amputation) was thought to promote the formation of a permanent fibrotic scar that prevented myocardial regeneration, whereas periostin was no longer localized in the wound at 30 days after amputation in zebrafish[28].

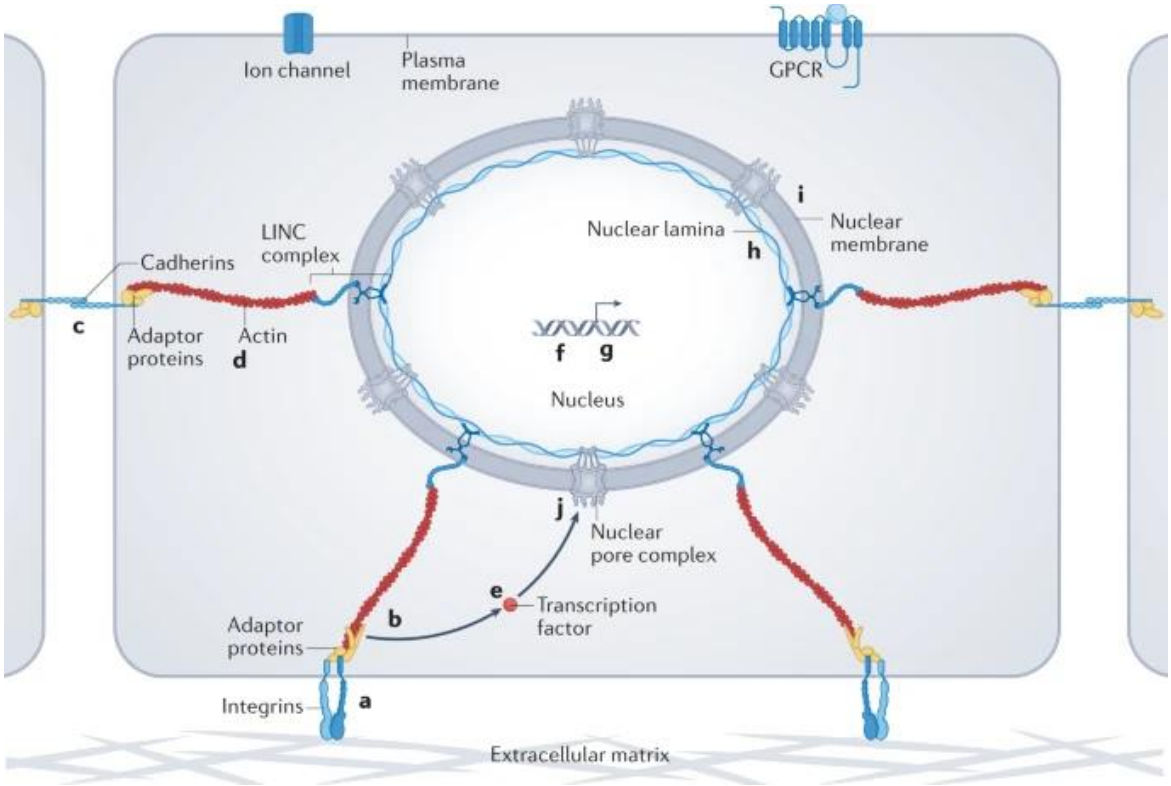
Remarkably, similar cellular and molecular mechanisms have been shown to determine the capacity of the mammalian heart to regenerate in the short time window immediately after birth[29]. The role of the ECM during the process of heart regeneration has received attention in the light of findings showing that decellularized ECM from regenerating zebrafish hearts imparted beneficial effects after injection into infarcted mouse hearts[30]. Similar benefits were also observed after injecting ECM from neonatal mouse hearts into infarcted mouse hearts[31], suggesting that ECM-related factors in the neonatal mouse heart and the regenerating zebrafish heart are involved in mediating cardiac regeneration. Comparisons of the ECM composition between regenerating and control zebrafish hearts or between neonatal and adult mouse hearts identified specific factors, such as hyaluronic acid, fibronectin, agrin and periostin, that were upregulated in the ECM of hearts with regeneration capacity and were functionally associated with the regenerative response[4,32,33,34]. However, given that these findings were generated from genetic loss-of-function and gain-of-function experiments, the relative contribution of changes in ECM composition and mechanical properties to the regenerative capacity is difficult to discern. Of note, direct measurements of myocardial ECM stiffness during cardiac regeneration in zebrafish revealed a marked but transient softening of the ECM that coincided with the peak in cardiomyocyte proliferation[35]. Consistent with this finding, the transition of the mouse neonatal heart from one with regenerative capacity to one without regenerative capacity is associated with a sharp increase in cardiac ECM stiffness[36] (Fig. 1). Both events seem to be causally related, given that reduction of ECM stiffness with β-aminopropionitrile, a pharmacological inhibitor of the ECM crosslinker enzyme lysyl oxidase

(LOX), extends the time window of regenerative capacity[36]. Whether a soft cardiac ECM acts as a key mediator of successful heart regeneration, or whether it merely facilitates a permissive environment for regeneration to occur, remains to be clarified. However, compelling evidence has shown that experimentally induced 'softening' of cardiac ECM dictates the disassembly of cardiomyocyte sarcomeric structures, thus re-establishing cardiomyocyte division[37]. Furthermore, interference with cardiac ECM hardening in vivo has been shown to prolong the cardiac regeneration window in newborn mice[36]. The administration of components of the fetal or newborn cardiac ECM (such as agrin) has also been shown to promote cardiac regeneration in adult mice after myocardial infarction by inducing cardiomyocyte proliferation[38,39]. Together, these findings strongly suggest that softening of the ECM after myocardial damage is a key mediator of cardiac regeneration[40].

Cell–matrix and cell–cell communications

The mechanisms described in the previous section suggest a link between the ECM remodelling activity of cardiac fibroblasts and the regenerative potency of the heart. These events seem to be tightly controlled by a mechanical framework involving contractile cells. In this context, the question of how force is translated into coordinated gene expression to facilitate ECM repair and induce pathological remodelling remains unanswered. At a cellular level, force is transmitted to cells from the ECM or neighbouring cells through specific transmembrane molecules (such as integrins and cadherins), focal adhesion molecules, G protein-coupled receptors (GPCRs), stretch-activated ion channels or cytoskeletal proteins, which can convert changes in external mechanical signals (including ECM composition and stiffness) into discrete signal transduction pathways[41,42] (Fig. 2). The binding of cells to the ECM is achieved mainly through integrin heterodimers, which recognize different ECM molecules. In cardiac fibroblasts, the α1β1, α2β1 and α11β1 integrins, among others, bind to collagen[43,44], whereas the β3 integrins bind to arginine–glycine–aspartic acid (RGD) motifs within, for example, fibronectin[45]. Integrins link the ECM to the cellular cytoskeleton through a series of adaptor proteins, connecting the integrin cytoplasmic tails to intracellular actin fibres. Integrins and their associated adaptor proteins cluster in structures known as cell–matrix adhesion complexes, of which several types exist. In fibroblasts, these complexes range from nascent adhesions that last for only seconds and are submicrometre in size, to stable micrometre-sized focal or fibrillar adhesions, which can last for minutes or hours and include dozens of different types of molecule[46]. Of note, cardiac cell types such as cardiomyocytes, cardiac fibroblasts or smooth muscle cells have specific types of cell adhesion complex, such as costameres, dense plaques and podosomes[47]. When force is applied to adhesion complexes, either by the cell itself through actomyosin contractility or by external deformation of the ECM, a mechanical signal is transmitted through both integrins and adaptor proteins, triggering a series of molecular events. For integrins, force application can trigger a switch from a bent to an unbent conformation, increasing their affinity for ECM ligands[48], promoting integrin clustering[49] or increasing the lifetime duration of ECM binding[50]. The last of these effects is caused by 'catch bond' behaviour, which is characterized by an increase in receptor–ligand bond lifetime (up to a certain value) in response to tensile force applied to the bond.

Fig. 2: Cellular force transmission and mechanisms of mechanotransduction.



Force is applied to cells either via the extracellular matrix through transmembrane proteins such as integrins, or via other cells through transmembrane proteins such as cadherin. When applied, force is transmitted all the way to the cell nucleus through the actin cytoskeleton, which is connected to integrins or cadherins via different adaptor proteins, and the linker of nucleoskeleton and cytoskeleton (LINC) complex. Force can trigger several types of cellular event: a, changes in integrin conformation and in the properties of binding to the extracellular matrix48,49,50; b, changes in adaptor protein conformation, triggering binding to other proteins51,52,254; c,d, changes in cadherin–cadherin binding properties and in the conformation of cadherin adaptor proteins57,58,59; e, downstream effects from a–d that release transcription factors bound to cytoplasmic partners, allowing the transcription factors to shuttle to the nucleus72,73,74; f, changes in nuclear mechanical properties that in turn tune the nuclear mechanosensing responses78; g, changes in the accessibility of chromatin, regulating transcription79,80; h, conformational changes in nuclear lamina proteins, leading to downstream effects81; i, changes in nuclear membrane tension, triggering signalling pathways82,83; and j, changes in the diameter and permeability of nuclear pore complexes, affecting the shuttling of transcription factors76. GPCR, G protein-coupled receptor.

For adaptor proteins, several force-induced events can occur concomitantly, thereby giving rise to complex intracellular signalling that is mediated by integrin-linked kinase, talin or focal adhesion kinase, all of which contribute to cardiac remodelling[51,52,53]. However, distinguishing the primary force-induced events from subsequent biochemical signalling is often challenging, particularly given that the expression levels of integrin subunits and their binding affinity for ECM components varies depending on pro-fibrotic and pro-inflammatory signalling[54]. A similar

conceptual framework can be applied to cell–cell adhesion complexes mediated by cadherins, which are also highly relevant in fibroblasts and myofibroblasts, in particular N-cadherin and OB-cadherin[55,56]. Cadherin–cadherin bonds across cells also have a force-dependent lifespan owing to the conformation of binding[57] and the link to actin through adaptor proteins such as α-catenin, which in turn can unfold under force[58]. Interestingly, α-catenin bonds to actin also behave as catch bonds[59].

Cardiomyocytes possess both desmosomes and costameres, which are specialized cell adhesion structures with essential roles in myocardial integrity and mechanical control. Desmosomes constitute the main intercellular adhesion complex in cardiomyocytes and facilitate the distribution of tensile strength in adjacent cells. Desmosomes normally connect the cardiomyocytes in series and are located on the short axis of the barrel-like cardiomyocyte structure in the so-called intercalated discs[60]. Although the main function of desmosomes is to maintain firm adhesive contacts between adjacent cardiomyocytes, a study has shown that these structures are subjected to the effects of strain[61]. Unlike desmosomes, costameres occupy the long axis of cardiomyocytes and act as specialized focal adhesion structures through which the contractile cells are connected to the surrounding ECM[62]. Costameres connect the sarcomeric Z-bands with the extracellular environment and have an important shock-absorbing function[47]. They are also involved in the sensitivity of cardiomyocytes to stiffness and participate in the stiffness-dependent maturation of the sarcomeric structures[63].

Finally, the intracellular transmission of mechanical cues in cardiac fibroblasts can be elicited through mechanically responsive ion channels[64,65]. Indeed, although cardiac fibroblasts are unresponsive to the action potentials that regulate coordinated cardiomyocyte contraction, they express a series of mechanically activated cation channels that can regulate various physiological functions and might be involved in cardiac pathologies. These mechanosensitive channels include members of the Piezo family, in particular Piezo 1, which is involved in stretch sensitivity and has been associated with atrial fibrillation[66], as well as transient receptor potential (TRP) channels such as TRPC3, TRPC6, TRPM7 and TRPV4. The association between these TRP channels and transforming growth factor-β (TGFβ)[67] in the context cardiac fibrosis has been documented[68,69,70].

Nuclear translation of mechanical forces

In addition to activating intracellular signal transduction, mechanical cues are also directly involved in transcriptional regulation, such as by mediating the shuttling into the nucleus of transcription factors or transcriptional regulators that are normally retained in the cytoplasm[71]. Such molecules can be released from cytoplasmic stores and allowed to shuttle into the nucleus in response to a mechanical stimulus occurring at the level of cell–cell or cell–matrix adhesions. For example, the interaction between β-catenin and E-cadherin is disrupted after the application of force, allowing β-catenin to shuttle into the nucleus and activate WNT signalling[72]. Similarly, myocardin-related transcription factor A (MRTFA) binds to monomeric actin (G-actin) but not to filamentous, polymerized actin (F-actin)[73,74], and, given that actin polymerization is one of the main effects of mechanosensing events at cell–matrix adhesions, MRTFA is released and subsequently enters the nucleus to activate transcription[74].

Alternatively, mechanosensing events can be triggered directly in the nucleus by forces transmitted from the actomyosin cytoskeleton via the linker of nucleoskeleton and cytoskeleton (LINC) complex[75,76], and also by extracellular forces, such as when cells deform to migrate across narrow constrictions[77]. The application of forces to the nucleus can have numerous consequences. First, forces on the nucleus can affect chromatin architecture, change nuclear mechanical properties and protect the nucleus against mechanical stress[78]. Furthermore, nuclear compression or strain forces can also increase the accessibility of the transcription machinery to increase gene expression[79,80], alter the conformation of nucleoskeletal proteins such as lamin[81] to trigger mechanosensing events, or unfold the nuclear envelope, leading to an influx of calcium and increased cell contractility[82,83]. Finally, transfer of cytoskeletal forces to the nucleus can also increase the diameter and permeability of the nuclear pore complexes[76,84,85,86], affecting the transport of transcriptional regulators. This mechanism has been documented for the transcriptional coactivator YAP[76], the major transcriptional effector of the Hippo pathway[87], and has also been shown for other transcriptional regulators, such as mothers against decapentaplegic homologue 3 (SMAD3), twist-related protein 1 or zinc finger protein SNAI1 (also known as SNAIL)[86,88,89]. Interestingly, nucleocytoplasmic shuttling depends not only on nuclear force, but also on the mechanical properties of the shuttling molecule itself[76,90], thus providing an elegant and complex mechanochemical control system (Fig. 2).

Cyclic strain[91] and ECM stiffness[92] directly promote cardiac fibroblast activation and proliferation through YAP activation. By contrast, inhibition of the Hippo pathway or activation of YAP promotes cardiomyocyte proliferation and improves cardiac function[93,94]. Accordingly, the increase in ECM stiffness after birth has been associated with inhibition of cardiomyocyte proliferation and an increase in cardiomyocyte hypertrophy, a process that is dictated by the Hippo-dependent phosphorylation of YAP[95]. The increase in ECM stiffness after birth has also been associated with YAP sequestering in the cytoplasm in association with the dystroglycan complex[39] or other components of the intracellular junctional complex (such as α-catenin)[96]. The profile and expression of integrins during cardiac development can also explain the crosstalk between ECM and cell response behaviour, including the shift from a proliferative to a non-proliferative state in cardiomyocytes through YAP and TAZ (transcriptional co-activator with PDZ-binding motif; also known as WWTR1) reversible nuclear translocation, which regulates genes related to cell cycle regulation and proliferation, such as CCNA2, CCNB1, CDC2, CDC25, AURKA and AURKB[97]. Likewise, a fibronectin-enriched ECM, produced by embryonic fibroblasts, promotes cardiomyocyte proliferation through the β1 integrin signalling pathway, whereas a higher collagen I density and crosslinking, together with the enrichment in collagen III and elastin, contributes to cardiomyocyte differentiation and lack of regenerative capacity of the adult heart[98]. Cardiomyocyte binucleation after birth has been shown to be dictated by a fibroblast-dependent, postnatal, non-permissive environment for cardiomyocyte mitotic rounding and cytokinesis, through the embryonically enriched ECM proteins slit homologue 2 protein and nephronectin[99], further supporting the role of cardiac fibroblasts and ECM modulation in cardiomyocyte homeostasis.

Given the effect of YAP co-transcriptional activity on cardiomyocyte proliferation, in vivo ablation of the Hippo pathway holds promise for cardiac regeneration[100,101] and repair[90]. However, the success of this strategy depends on the developmental stage and the cell types

that are targeted. Indeed, although YAP activity in cardiomyocytes promotes cardioprotection[102,103] and cardiogenesis[104], its activation in cardiac fibroblasts might trigger their transition towards the myofibroblast phenotype in diseased adult hearts to induce pro-fibrotic effects[105]. Furthermore, the reactivation of YAP at the border zone of the infarction seems to be a physiological response to ECM remodelling[102] and local mechanical stress[106], which promotes cardiomyocyte survival and induces their re-entry into the cell cycle[93.] This effect is linked to the upregulation of YAP transcriptional targets involved in cell cycle progression and cytoskeletal protein expression[107] downstream of dystroglycan 1 (ref.[108]), neuregulin 1–ERBB2 (ref.[109]) or agrin[39] signalling axes.

Mechanical, inflammatory and metabolic cues

Biophysics of ECM remodelling

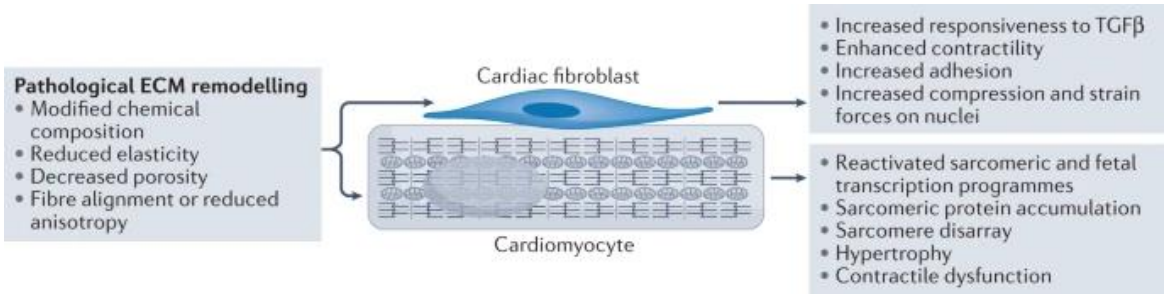
The response of the adult heart to pathogenic insults is essential to preserve the integrity of the myocardial wall from rupture and is characterized by the rapid deposition of a fibrotic matrix that can alter the mechanical properties of the myocardium[110]. As a result, myocardial stiffness increases, which is considered an important prognostic and diagnostic parameter of reduced diastolic left ventricular (LV) function[111]. Animal model studies have indicated that the loss of cardiomyocytes after myocardial infarction is compensated for by a fibrotic scar that is stiffer than healthy myocardium (Young's modulus of a pathological heart is in the range 35–100 kPa; that of a healthy heart is in the range 10–15 kPa)4. Aged animals[112,113] and animals subjected to metabolic stress[114] have increased interstitial fibrosis and ventricular stiffness, suggesting that these risk factors might have a role in cardiac stiffening caused by pathological changes in fibroblasts[115]. In humans, end-diastolic myocardial stiffness (measured by shear wave imaging) increases steadily with age as the result of age-induced physiological myocardial fibrotic growth and ECM fibre disarray[116]. An increase in myocardial stiffness has also been shown in hearts that have undergone pathological remodelling, such as the hearts of patients with hypertrophic cardiomyopathy[116]. A study that assessed decellularized tissues taken from patients diagnosed with end-stage heart failure and eligible for LV-assist device (LVAD) implantation or organ transplantation showed that ECM from dilated or ischaemic hearts consistently and reproducibly loses complexity, most likely as a result of the excessive deposition of aligned and compact fibre bundles instead of helical structures[42]. The severe rearrangement of collagen fibres and the consequential loss of structural complexity are linked to diminished ECM interconnected porosity[42].

The increased collagen deposition and the mechanical consequences associated with pathological ECM remodelling directly hinder cardiomyocyte contractile function[117]. Indeed, the generation of tension by the actomyosin molecular motors on cell–cell and cell–matrix contacts creates an excessive strain on the sarcomeres owing to the reduction in ECM elasticity and an increase in spring-like behaviour[117]. Cardiomyocytes perceive substrate rigidity through a multimodal mechanosensing machinery guided by the combined activity of non-muscle and muscle myosin, and operated through the cyclic or continuous stretching of the mechanosensitive protein talin at the costameres[118]. Age-related and pathology-induced modifications in the expression of costameric proteins have been attributed to cardiomyocyte cortical stiffening and cardiac dysfunction in aged[48] and diseased[51,119,120] hearts. Of note,

increased levels of vinculin, a mechanosensitive protein involved in the linkage of integrin adhesion molecules to the actin cytoskeleton, were detected in animal models and humans, together with a switch in integrin subunit expression, which might lead to changes in cardiomyocyte mechanosensation[121,122,123]. Another mechanism by which cardiac ischaemia, age or metabolic stress might affect cellular mechanosensation in the diseased heart is collagen crosslinking by non-enzymatic pathways (such as those mediated by the accumulation of advanced glycation end products)[124] and enzyme-dependent pathways (such as those mediated by LOX enzymes)[125]. Importantly, experimental studies have shown a crosstalk between the fibrotic pathway mediated by TGFβ and the expression of LOX in ischaemic and aged mice, thus establishing a mechanoparacrine paradigm in the maladaptive remodelling of the diseased heart[126,127,128].

Finally, the changes in ECM mechanics and the intracellular sensing of mechanical cues also have an effect on cardiomyocyte phenotype and mechanical performance[129,130]. Indeed, similar to the stiffness-dependent maturation of the contractile apparatus in terminally differentiated cardiomyocytes (such as those at the neonatal stages or after differentiation from induced pluripotent stem cells[117,131,132]), pathological ECM stiffness induces defects in myofibril organization that result in changes in mechanical output, similar to those caused by excessive stretch. As an adaptive response, the contractile apparatus of LV cardiomyocytes is extensively remodelled and their geometry and contractility are changed[131,133]. For example, in the hearts of patients with heart failure with reduced ejection fraction, the contractile cells become extremely elongated with a ratio of length to width that is higher than the physiological ratio of 7:1, and there is a generalized thinning of the myocardial wall[134] (Fig. 3). By contrast, cardiomyocytes from patients with heart failure with preserved ejection fraction acquire a more compact shape that is characterized by a reduction in the ratio of length to width that is <7:1 and is accompanied by myocardial wall thickening and a decrease in LV diastolic volume[134].

Fig. 3: Effects of ECM remodelling owing to ageing or compensatory mechanisms on local variations in stiffness.



Pathological extracellular matrix (ECM) remodelling is prompted by a shift in the chemical composition of the local ECM, which results in reduced ECM compliance and interconnected porosity[42,111]. The resulting fibre alignment mediates a decrease in bulk anisotropy. ECM remodelling induces cell-specific effects on cardiomyocytes and cardiac fibroblasts, the main components of the adult heart. In response to pathological ECM remodelling, the responsiveness of cardiac fibroblasts to pro-fibrotic stimuli, such as transforming growth factor-β (TGFβ), is increased, their adhesion and contractility properties are strengthened, and they align. In cardiomyocytes, pathological ECM remodelling induces the reactivation of sarcomeric and fetal transcription programmes, which eventually leads to the hypertrophic phenotype characterized by sarcomeric disarray and contractile dysfunction.

Crosstalk between mechanical cues and humoral signalling

In the normal adult heart, the ECM is continuously subjected to the effects of compression and strain forces caused by cardiac motion. Therefore, a major function of cardiac fibroblasts involves the restoration of the ECM to a homeostatic state. Under pathophysiological conditions, such as ischaemia, pressure overload, inflammation, ageing and altered metabolism, cardiac fibroblasts proliferate and differentiate into myofibroblasts[7,8,110]. Although the shift of these cells towards a pro-fibrotic phenotype has been associated with biohumoral signalling (such as inflammation), studies in the past 20 years have shown that cell mechanics could also be involved. For example, cardiac fibroblasts cultured on stiff polyacrylamide substrates or exposed to static or cyclic mechanical loads adopt a myofibroblast-like phenotype that is characterized by increased α-smooth muscle actin fibre formation, ECM production, cell spreading and proliferation[3]. Although cardiac fibroblasts have not yet been shown to respond to strain-dependent signals in the setting of myocardial remodelling, a study showed that fibrotic tissue might form in an anisotropic pattern and according to specific lines of deposition that correspond to the distribution of the main ventricular strain forces[135], possibly connected to direct activation of YAP signalling by strain or compression forces[136]. Given that cardiac fibroblast proliferation can be activated by uniaxial strain[91], chronic activation of these cells, as observed in myocardial scarring, might be the result of geometry-sensing that occurs during the dynamic remodelling process[136].

In addition to the direct effects of mechanical cues on cardiac fibroblasts and progressive ECM stiffening[3,110], pro-fibrotic activity can be initiated via paracrine signalling from surrounding cells such as cardiomyocytes, endothelial cells, pericytes and recruited immune cells. This activation involves the release of fibrogenic mediators including growth factors such as TGFβ[137], neurohumoral factors such as noradrenaline[138], angiotensin II[139] and aldosterone[140], and matricellular proteins such as thrombospondin 1 (ref.[141]) and osteopontin[142], which bind to GPCRs (including the β-adrenergic and angiotensin II receptors) and matricellular receptors, respectively.

Cardiac fibroblasts and myofibroblasts participate in the inflammatory response at different levels[7,8,143]. They can induce the release of cytokines[144] and chemokines[145,146,147], which are important for the recruitment of specific immune cells to the heart. The specific cytokine composition has in turn been shown to modulate the chemokine secretion profile of cardiac fibroblasts and, consequently, the recruitment of pro-inflammatory or anti-inflammatory monocytes[146]. Furthermore, mechanical stress caused by stretching of cardiac fibroblasts can also trigger chemokine release[3]. Cardiac fibroblasts and myofibroblasts can activate the expression of adhesion molecules on the endothelium[3], as well as facilitate the migration of monocytes through the basolateral membrane via the stimulation of gelatinase expression3. Finally, cardiac fibroblasts can also activate the NLRP3 inflammasome and trigger the release of IL-1β[148]. The link between mechanosensation and the fibro-inflammatory potential of cardiac

fibroblasts has been strengthened by the observation that the conditioned medium from cultured cardiac fibroblasts deficient in YAP and TAZ has an inhibitory effect on the migration of bone marrow-derived macrophages in vitro compared with the conditioned medium from control fibroblasts, and results in macrophage polarization to an M2 phenotype[149].

Immune cells can in turn be activated by mechanical stress and thus contribute to myocardial remodelling[150,151]. Inflammatory cells are triggered by neurohumoral signalling, such as the renin–angiotensin II–aldosterone pathway[150,152] or the β-adrenergic receptor pathway[153], to drive fibrogenesis. For example, splenocytes from mice with heart failure induced by angiotensin II infusion have been shown to induce more collagen production in fibroblasts than splenocytes from control mice[139]. Of note, activation of GPCR kinase 2 (GRK2) in cardiac fibroblasts might transduce, at least in part, the fibrogenic actions of β-adrenergic receptors in the infarcted myocardium[154,155] after YAP or TAZ signalling[156], and increased GRK2 levels in the heart can correlate with the levels of circulating lymphocytes[157]. YAP activation in macrophages can influence macrophage polarization, resulting in a pro-inflammatory and impaired reparative response after myocardial infarction[158]. This response includes activation of the NLRP3 inflammasome via inhibition of the proteasomal degradation of NLRP3 (ref.[159]). Whether altered cell mechanics and YAP signalling involve IL-1β expression (as reported in gastric carcinogenesis[160]) or NLRP3 activity in cardiac fibroblasts remains unknown. Finally, a study has shown that immune cells can respond to YAP and TAZ signalling and might blunt the fibrotic response in the heart[161]. YAP and TAZ signalling in the epicardium regulated an immunosuppressive response after myocardial infarction in mice by attracting regulatory T cells, and this process was associated with a decrease in fibrosis[161].

In addition to the crosstalk between mechanical and pro-inflammatory cues, an interplay between mechanics, the microenvironment and cellular metabolism also exists. The conversion of fibroblasts to excitable myofibroblasts, which have increased contractility and higher synthetic and secretory capacity, is characterized by an increase in cellular energy demand[162] and a rise in mitochondrial content and cellular respiration[163]. In line with the observation that patients with heart failure receiving statin therapy have fewer cardiac myofibroblasts than patients with heart failure who are not receiving statin therapy[164], statin administration has been shown to trigger the dedifferentiation of myofibroblasts into fibroblasts in vitro, which is associated with a reduction in cellular respiration[164]. Metabolic dysfunction, as present in patients with heart failure or diabetic or obesity-related cardiomyopathy, might lead to increased cardiac fibrosis and altered ECM mechanics[165,166,167]. In this regard, growing evidence shows that AMP-activated protein kinase, a key enzyme involved in cellular energy metabolism that is reduced in heart failure[168,169], can promote the phosphorylation of YAP, limiting YAP-driven transcriptional regulation[170].

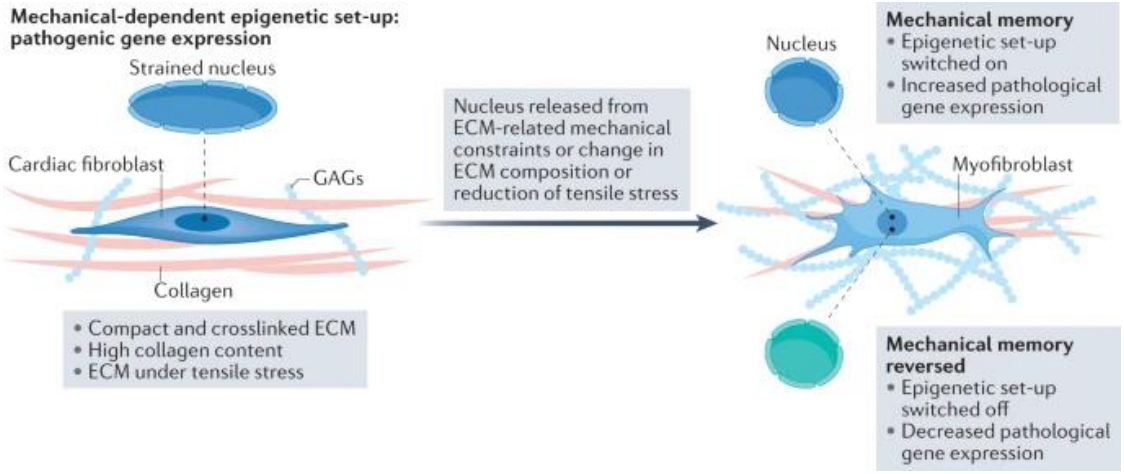
Mechanical ‘memories’ and cellular commitment

The cardiovascular system is subjected to the effects of modifiable and unmodifiable risk factors, such as hypercholesterolaemia, hypertension and ageing, which are known to induce permanent modifications in gene expression through DNA methylation and post-translational modifications of the histones[171]. Given that these alterations can result in a chronic

upregulation of pro-inflammatory and pro-fibrotic signalling in the heart[172], they contribute to the acceleration of cardiac senescence and ageing. Likewise, modifications in the mechanical characteristics of the ECM, including compliance, viscosity and elastic behaviour, can permanently affect the activity of the transcriptional components of the cellular mechanosensing machinery, such as the YAP–TAZ complex, resulting in chronic activation of pathological pathways[173]. Given that mechanical cues can modify epigenetic marks6, and epigenetic modifications are transmitted throughout DNA replication and cell division[174], alterations in the mechanical characteristics of the ECM owing to tissue injury might establish feedback loops that contribute to permanent tissue damage and scarring, as in cardiac fibrosis[175]. In addition to potential epigenetic memory effects resulting from direct exposure of cells to metabolic alterations and mechanical cues, an important crosstalk between cell metabolism and mechanical sensing involves the direct susceptibility of mechanically activated molecular triggers to metabolically driven effects[156,176]. For example, the transcriptional activities of the YAP–TAZ complex, MRTFA and β-catenin are directly regulated by lysine acetylation[177,178,179], linking cell mechanosensation to the machinery that controls genome-wide chromatin activity, with possible cumulative effects on ageing of the myocardium[180,181,182]. This finding is of particular interest given that treatment with drugs with global epigenetic effects might restore the normal behaviour of cells by re-establishing typical responses to mechanical cues[183].

A final mechanical memory effect that could drive cellular evolution towards chronic pathogenic phenotypes is the change in the composition of the nuclear lamina or even the variation in heterochromatin and euchromatin content, which might trigger a persistent pathological transcriptional activation[184,185]. For example, a stable change in the composition of the nuclear lamina has been observed in aged cells compared with young cells, with the substitution of lamin B expression (prevalent in young cells) with lamin A or lamin C expression (prevalent in aged cells)[81]. Given that lamin composition in the nuclear envelope depends on the compliance of the ECM surrounding the cells and is linked to cellular senescence, hardening the ECM in the failing heart can potentially result in the permanent activation of pro-fibrotic genes by directly increasing nuclear rigidity, reducing the chromatin spring behaviour and thereby diminishing overall cellular plasticity[186]. Further studies are necessary to establish the link between chromatin mechanics and permanent epigenetic control of pathological gene expression in cardiac fibroblasts (Fig. 4).

Fig. 4: Mechanical 'memory' underlies chronic pathological cellular phenotypes.



Mechanical 'memories' underlying chronic pathological cellular commitment in the myocardium are the result of pathological strain forces. Cells in contact with a stiff extracellular matrix (ECM) or with ECMs aligned in geometric patterns acquire specific gene expression set-ups that might give rise to 'memory' effects[173,175]. Cells transiently exposed to 'stiff' environments and re-exposed to compliant ECMs do not lose this pathological set-up even when the traction forces deforming the nucleus cease to pull on the nuclear envelope. This phenomenon is called mechanical memory, is set up at an epigenetic level, and has potentially permanent consequences for the activation of pathological and cellular ageing signalling in the fibrotic heart, leading to heart failure. Strategies to reverse mechanical memory leading to downregulation of pathological gene expression are currently under investigation. GAG, glycosaminoglycan.

3D culture systems to mimic cardiac remodelling

During cardiac fibrosis and adverse cardiac remodelling, quiescent cardiac fibroblasts differentiate into ECM-depositing myofibroblasts via a combination of biochemical and mechanical stimuli[110], but the molecular mechanisms involved in fibrotic growth remain poorly understood. In the healthy human myocardium, cardiac fibroblasts remain quiescent during contraction despite the fact that continuous mechanical stimulation should ultimately activate pro-fibrotic mechanotransductive pathways[11]. This observation supports the hypothesis that cardiac fibroblast activation occurs only in response to combined mechanical activation and biohumoral stress signalling. Conventional 2D cell culture does not allow the assessment of such complexity given that plastic or matrix adherence can mechanically activate the fibroblasts, and the mechanical cues in animal models are not well characterized. 3D culture platforms have therefore emerged in the past 25 years as alternative models to conventional 2D cell culture to better resemble the endogenous environment of myocardial tissues, including ECM stiffness, cyclic mechanical strain and other relevant biochemical cues. The 3D culture platforms range from simple cardiac organoid structures[187,188] to engineered heart tissues[189] or fully recellularized myocardial tissues[190].

Given that cells exert a certain level of traction on the surrounding ECM, which is established through integrins that are physically connected to the ECM and the cytoskeleton[9], the mechanical properties of cell culture ECM substrates used to study fibroblast behaviour under pathological conditions must be properly controlled to direct cell phenotype[191]. In addition, when possible, these mechanical properties should be characterized[192]. For example, by using a

3D cell culture model consisting of cardiomyocytes and cardiac fibroblasts encapsulated within a mechanically engineered gelatin methacryloyl hydrogel[193] or a 3D system consisting of cardiac fibroblasts in methacryloyl hydrogels containing different substrate stiffnesses[194], myofibroblast behaviour and differentiation could be altered to maintain a more quiescent phenotype and rendered more responsive towards pro-fibrotic triggers such as TGFβ[195]. These findings confirm the results obtained by culturing cells in hydrogels with reversible stiffness, which show that changing the Young's modulus from ~32 kPa to ~7 kPa reverses myofibroblast activation[196].

The principal force that fibroblasts experience in the heart is the strain generated by myocardial contractility. Mechanical loads can vary to comply with pumping forces during cyclic contractions, which are approximately 1 Hz in humans and 10 Hz in mice[197]. In addition to cyclic rhythmicity, cells experience forces from myocardial stiffness. Although matching in vitro substrate stiffness to the stiffness of the myocardium will improve the usefulness of cell culture platforms, static tension does not match the cyclical mechanical stress exerted by the myocardium. Indeed, although the effects of 2D cultured cardiomyocytes on cyclic strain have been assessed under different conditions, the direct effects of cyclic strain on fibroblasts and vice versa require further study[198,199]. Neonatal rat fibroblasts show increased production of collagens, fibronectin and glycosaminoglycans after 24 h of cyclic stress[200], which is mediated by increased p38 mitogen-activated protein kinase signalling[201]. However, in another study, exposing cells to 72 h of 10% stretch did not alter TGFβ-induced phosphorylation of SMAD2 and ERK[202]. These discrepant findings are difficult to compare because different levels of strain and frequencies were used, necessitating standardized parameters and protocols for fibrosis studies. Interestingly, different ECM subtypes provide variable cellular responses to mechanical cyclic stretch and TGFβ signalling[203]. Simple topological changes in cardiac fibroblast organization are sufficient to induce chromatin remodelling and global changes in gene expression[10]. Finally, diverse cell sources might contribute to the observed differences, because expanded adult, but not fetal, cardiac fibroblasts in vitro can deteriorate the electrical and mechanical function of co-cultured cardiomyocytes[204].

Studies investigating the effect of mechanical strain on cardiac fibroblasts are scarce and generally show contradictory results[200,202]. The reasons underlying these mixed findings might include discrepancies in the strain modes and magnitudes being tested, the fibroblasts assessed (such as neonatal versus adult, rodent versus human, the region where the fibroblasts reside or patient-specific factors), the biological binding sites and the mechanical characteristics of the ECM substitute, which all affect the behaviour of cardiac fibroblasts. Controlling the microenvironment of cardiac fibroblasts is, therefore, essential when assessing the mechanistic pathways underlying their behaviour and their therapeutic potential. The inclusion of other cell types (including cardiomyocytes, endothelial cells or immune cells) in addition to cardiac fibroblasts in model systems will provide a more in-depth assessment of the cellular response of cardiac fibroblasts to mechanical strain. Furthermore, ultrathin (100–400 μm) sections of living myocardial tissue in which the native multicellularity, architecture and structure of the heart is maintained can provide information at the tissue level, and can serve as a uniquely controlled electromechanical system in which the bidirectional crosstalk between mechanical activity and electrical excitation can be quantitatively monitored or evaluated. These slices can be prepared from human specimens and have proven to be a clinically relevant multicellular human model and screening platform[205] for translational

cardiovascular research[206]. Studies assessing further evolutions of 3D culture, including the use of 3D printing and the development of engineered cardiac macrotissues[207], are ongoing with the ultimate aim of achieving an in vivo setting that mimics the complex structure of the ECM and various cell types in myocardial tissue, as well as the physiological function of the heart. The current limitations of culturing cells in 3D platforms for heart failure drug screening (such as low throughput) can be overcome by inclusion of sensors in the bioreactor platforms or the tissue constructs, the adoption of algorithms for high-throughput image analysis applied to advanced microscopy and imaging[208], and the use of machine learning and artificial intelligence for high-throughput capture of cellular features[209].

Mechanotherapeutic strategies

Reversing ECM stiffening in the remodelled heart

The heterogeneity of fibrotic processes in the heart and the multiplicity of the involved molecular effectors warrant caution regarding the possibility of using similar cardioprotective strategies for all types of pathological myocardial remodelling[210]. However, the biunivocal relationship between the physical properties of cardiac ECM and the pro-fibrotic activity of resident cardiac fibroblasts has prompted the development of new cardioprotective approaches that are based on the modulation of cardiac ECM biochemical and biophysical properties. Attempts in this direction have been made using pharmacological inhibitors, antibodies or even ECM components to target effectors of collagen crosslinking or to reduce cardiac ECM stiffening[124]. For example, investigators have used a combination of pharmacological and genetic approaches to show that the inhibition of the crosslinking enzyme LOX homologue 2 has a protective effect against the progression of diastolic dysfunction in a transverse aortic constriction mouse model through a reduction in TGFβ–AKT signalling[128]. Furthermore, injection of agrin (a component of the fetal or neonatal cardiac ECM) into the heart in adult mice subjected to myocardial infarction caused a partial reactivation of cardiomyocyte proliferation owing to the disassembly of sarcomeres and the release of YAP transcription factor from the dystrophin–glycoprotein complex[39]. Inhibitors of RHO-activated kinase, TGFβ receptor and YAP (verteporfin) have potent anti-fibrotic effects that result in the reduction or reversion of myofibroblast activity and restoration of ECM mechanical function[211,212,213]. However, given the ambivalent function of YAP signalling in cardiac fibroblasts and cardiomyocytes (discussed below), the targeting of YAP in the pro-fibrotic cells of adult pathological hearts to reduce fibrosis and induce cardiomyocyte division[214,215,216] should be conducted using cell-specific strategies, or tamoxifen-inducible Cre recombinase or CRISPR–Cas9 gene editing vectors that take advantage of the selectivity of cardiac fibroblast-active promoters (such as COL1A2 or TCF21)[149,212]. Furthermore, sophisticated delivery systems such as nanoparticles can selectively target these cells via antigen recognition. Pharmacological inhibition of αV integrin has also been shown to reduce the development of cardiac fibrosis and improve cardiac function after myocardial infarction[136,217]. Together, these findings suggest that acting on the molecular machinery that is involved in sensing the biophysical characteristics of the ECM surrounding contractile and non-contractile myocardial cells might result in the possible reversion of fibrotic progression caused by acute or chronic myocardial insults or other risk factors. Such approaches have the potential to reactivate myocardial regeneration programmes that were lost in the transition from the neonatal to the postmitotic cardiac developmental stage, arguably owing to mechanical cues.

Mechanical unloading and reverse remodelling

The benefits of mechanical unloading in the context of acute myocardial infarction before reperfusion to reduce infarct size[218], in fulminant myocarditis[219] and in chronic heart failure to promote myocardial recovery and remission[220], are currently under investigation. Such findings add to the body of evidence on the biology of heart failure and the potential for myocardial reverse remodelling[221], remission[222] and recovery[223] provided by the assessment of patients with an LVAD[220]. Insights from human explanted hearts and studies comparing paired pre-LVAD and post-LVAD myocardial tissue samples have consistently demonstrated that ventricular structural and functional improvements are accompanied by favourable changes in the myocardium at the cellular and molecular levels[220]. These changes include regression of cardiomyocyte hypertrophy[224], improved calcium handling[225], mitochondrial ultrastructure[226] and cytoskeletal organization[227], re-entry of cardiomyocytes into proliferation[228] and increased microvascular density[229], although these positive effects were not associated with changes in[230] or development of[231] myocardial fibrosis. Of note, patients with less cardiac fibrosis at the time of LVAD implantation are more likely to have improvements in ejection fraction and to recover while receiving device support[232,233] than patients with extensive fibrosis. Indeed, the likelihood of a patient recovering after LVAD implantation is determined by the underlying pathophysiology, such as non-ischaemic aetiology, shorter duration of heart failure and smaller LV size[234,235,236]. Importantly, a full recovery or reverse remodelling cannot be attained after LVAD implantation, which follows on from findings in patients with an LVAD indicating that only a small proportion of heart failure-dysregulated genes normalize with LVAD support, with a higher percentage of gene normalization in non-ischaemic than in ischaemic heart failure[237,238].

Reduced integrin expression, accompanied by decreased mRNA levels of innate immune response components, such as protein S100A8 and S100A9 and NLRP3, lower numbers of immune cell infiltrates, changes in ECM and improved recovery were observed after prolonged mechanical unloading (PROPELLA concept) via an axillary pump in fulminant myocarditis[219,239]. These study findings further support the link between mechanical stress and cardiac inflammation and the capacity of mechanical LV unloading to blunt these processes in this specific clinical phenotype. Future research is required to determine LV unloading efficacy (bridge-to-recovery or remission versus bridge-to-transplantation) and the role of the ECM and activation of mechanosensitive pathways in this context. The use of animal models with reversible cardiomyopathy[220], including aortic banding–debanding[240] and heterotopic cardiac transplantation[241] models, could facilitate further mechanistic insights into the biology of myocardial recovery and the underlying tissue mechanics.

Future perspectives

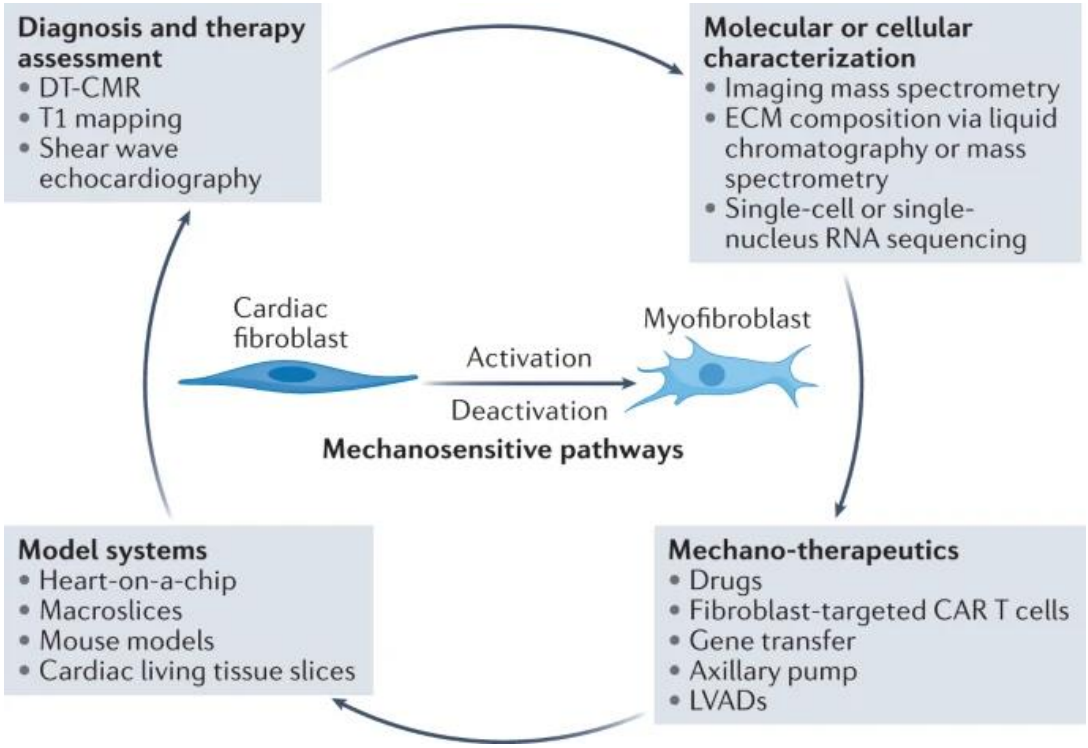
As discussed above, convincing evidence shows that the mechanical characteristics of the myocardium are more than a coincidental feature and have an important role in dictating crucial cellular responses involved in cardiac morphogenesis, maturation and pathology. Although the processes of cardiomyocyte maturation and fibrosis have long been viewed as irreversible events, increasing evidence suggests that a reversion might be possible, provided that the mechanical features of the environment surrounding the contractile and non-

contractile cells are restored to their native conditions. As discussed in this Review, a mechanical 'plasticity' of the cardiac ECM is naturally present in amphibians and fish, in which the milder mechanical workload of the heart, possibly owing to the lesser effect of gravitational forces involved in amphibious or aquatic living, seems to allow for the ECM to be restored to native conditions by a generalized softening after damage for the cardiomyocytes to dedifferentiate and re-enter the cell cycle, even in adulthood, boosting cardiac regeneration[23,24,25,26]. Conversely, the mechanical conditions occurring as a consequence of risk factor exposure or acute or chronic injuries to the heart seem to exacerbate the opposite response, which favours scarring, matrix compaction or stiffening and exposure of cardiomyocytes to forces that are not permissive for cell division but promote their pathological remodelling[175]. Whether restoration of mechanically permissive conditions for regeneration in the human heart (by, for example, inducing partial depolymerization of sarcomeric components and promoting YAP nuclear translocation in cardiomyocytes, or blocking or reducing YAP transcriptional activity in cardiac fibroblasts) is sufficient to promote cardiac regeneration is still under investigation.

Although cardiac fibrosis is the best predictor of heart failure and of a poor prognosis in patients with heart failure, clinical trials testing anti-fibrotic therapies that target traditional pathways have been disappointing so far[242]. This lack of success can be ascribed to the heterogeneity of heart failure, the diversity of fibrosis, the variation in ECM composition depending on the fibrogenic stage, and the heterogeneity in the activity of cardiac fibroblasts. The lack of a good understanding of how cardiac fibroblasts and cardiomyocytes are able, in concert, to detect and interpret the mechanics of the tissue where they exert their function seems to be a major limitation in the resolution of the problem. To overcome these limitations, a more thorough assessment of the fibrotic stage (refined diagnosis), the use of target-specific treatment strategies and an improved longitudinal follow-up of the effects of treatments are required. Improvements in non-invasive cardiac MRI via T1 mapping have enabled detection of diffuse fibrosis, in contrast to standard late gadolinium enhancement techniques that allow accurate detection only of regional scarring and focal fibrosis[243]. In addition, the use of diffusion tensor imaging enables the assessment of the microstructure of the heart[244]. Furthermore, myocardial stiffness can be determined with shear wave imaging via echocardiography[116]. Imaging mass spectrometry on endomyocardial biopsies could be of help in assessing region-specific changes in the ECM[219,245], whereas the matrisome can be assessed via liquid chromatography mass spectrometry[246]. Characterization of fibroblast subsets can be achieved via single-cell and single-nucleus RNA sequencing[2,10], whereas computational algorithms based on single-cell transcriptomics[247] can provide insights into spatial information to study tissue organization and spatial gene expression patterns. These novel techniques will lead to improved assessment of fibrosis and will provide important avenues for early drug intervention before irreversible fibrosis takes hold. Beyond the early diagnosis, refined, topological characterization of fibrosis or ECM modulation and fibroblast state, influenced by mechanosensitive pathways, might lead to potential novel therapeutic targets and the use of repurposed or novel therapeutic strategies, including new technologies for drug delivery (such as nanoparticles and small peptides) and advanced fibroblast-specific targeting strategies (such as gene transfer and chimeric antigen receptor T cells[248,249]). Furthermore, future studies should confirm the use of approaches that are based on modulation of the biochemical and biophysical properties associated with the ECM, including LV unloading strategies. Finally, characterization of fibroblast subtypes will help to further define the fibro-inflammatory

response, as has been shown in other diseases such as arthritis[250], and might be the key for blunting chronic inflammatory processes. The efficacy of newly identified therapeutic strategies might be assessed in optimized and (ideally) patient-specific model systems that take into consideration mechanical strain[206,209]. The use of such therapeutic approaches might be in turn optimally followed up by the improved imaging strategies and in-depth ECM and fibroblast characterization (Fig. 5).

Fig. 5: Integration of diagnostic and experimental pipelines for the evaluation of cardiac mechanotherapeutic approaches.



Advanced non-invasive cardiac imaging strategies, including diffusion tensor cardiovascular magnetic resonance (DT-CMR) imaging[244], T1 mapping[243] and shear wave echocardiography[116], allow improved assessment of cardiac remodelling and stiffness compared with standard late gadolinium enhancement techniques. Spatial characterization of proteomic signatures can be obtained via imaging mass spectrometry[219,245] and in-depth analysis of extracellular matrix (ECM) or matrisome composition via liquid chromatography or mass spectrometry[246]. Fibroblast subtypes can be characterized via single-cell and single-nucleus RNA sequencing, and computational algorithms based on single-cell RNA sequencing[247] can provide insights regarding spatial information to study tissue organization and spatial gene expression patterns. The above-mentioned techniques allow the refined diagnosis of the cardiac status reflecting the spatial ECM composition and fibroblast state, which is influenced by mechanosensitive pathways (from regional changes, such as ECM remodelling, fibrosis or stiffness, to structural factors such as ECM composition and, finally, to cellular factors such as fibroblast subtypes). These analyses can further lead to the development of novel mechanotherapeutics, including novel drug delivery systems (such as nanoparticles and small peptides) and advanced fibroblast-specific targeting strategies (such as gene transfer and chimeric antigen receptor (CAR) T cells), and can confirm the efficacy of approaches that are based on modulation of the cardiac ECM, including left ventricular unloading strategies (auxillary pumps or left ventricular assist devices (LVADs)). The efficacy of novel therapies can be tested in patient-specific model systems[206,209], and might in turn be better followed up using improved imaging strategies and in-depth ECM and fibroblast characterization.

Conclusions

In summary, more efforts should be directed towards understanding how the fundamental integration of mechanosensitive pathways affect the response of cells in a cardiac pathological context to facilitate a reversion of their maladaptive phenotypes. To what extent the pathological cardiac phenotype can be overridden by interfering with these pathways is not known. However, inhibiting fibrotic growth with strategies based on an increased knowledge of the mechanical control of cardiac fibroblast biology will, at the very least, block or retard the progression of fibrosis and, thus, reduce the burden of heart failure.

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Competing interests

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