FOCUS: CARDIAC REGENERATION

The interstitium in cardiac repair: role of the immune–stromal cell interplay

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Abstract | Cardiac regeneration, that is, restoring the original structure and function in a damaged heart, differs from tissue repair, in which collagen deposition and scar formation often lead to functional impairment. In both scenarios, the early onset inflammatory response is essential to clear damaged cardiac cells and initiate organ repair, but the quality and extent of the immune response varies. Immune cells embedded in the damaged heart tissue sense and modulate inflammation through a dynamic interplay with stromal cells in the cardiac interstitium, which either leads to recapitulation of cardiac morphology by rebuilding functional scaffolds to support muscle regrowth in regenerative organisms, or fails to resolve the inflammatory response and produces fibrotic scar tissue in adult mammals. Current investigation into the mechanistic basis of homeostasis and restoration of cardiac function has increasingly shifted focus away from stem cell-mediated cardiac repair towards a dynamic interplay of cells comprising the less regarded interstitial compartment of the heart, offering unexpected insights into the immunoregulatory functions of cardiac interstitial components and the complex network of cell interactions that must be considered for clinical intervention in heart diseases.

[H1] Introduction

Organismal survival depends on the maintenance of tissue integrity, calling upon developmental pathways to retain original tissue form and function. Throughout the animal kingdom, integral components of the tissue stroma choreograph embryonic development and pattern formation, such as the expression of the HOX family of patterning genes, determinants of body segmentation during embryonic development, by stromal cells[1,2]. In the highly regenerative axolotl, stromal cells have also been implicated in the establishment of limb polarity during regeneration[3]. In heart, cells such as fibroblasts, pericytes, endothelial and smooth muscle cells occupy the interstitium, which has now been redefined as a previously unappreciated fluid-filled interstitial space that drains to lymph nodes and is supported by a complex network of thick collagen bundles, lined on one side by fibroblast-like cells[4].. An effective response to tissue damage requires sequential phases of inflammation, tissue replacement, and maturation, orchestrated by a dynamic interplay between stromal and resident immune cells (Fig. 1). Rapid resolution of the pro-inflammatory phase and transition to a tissue reconstruction programme is a critical feature of regeneration, and perturbation of inflammatory resolution might delay repair after injury and aggravate degenerative diseases. The balance between regeneration and fibrotic repair varies between tissues, species, and developmental stages: tissue damage either elicits a recapitulation of organ structure and function, or leads to fibrosis, scarring, and organ failure[5,6].

Nowhere is the requirement for functional organ maintenance more critical than in the human heart, which beats about 3 billion times in a lifetime, pumping high-pressure blood throughout the body. This demanding pump function is achieved by cardiomyocytes, highly specialized muscle cells that depend on the support of a network of stromal cells including vasculature, nerves, and fibroblasts, as well as immune cell lineages. Extensive cell-cell interactions amongst the major cardiac cell types are established during development, and these initial interactions hold essential clues to cellular functions in the adult cardiac interstitium. For example, both fibroblasts and macrophages are electrically coupled with cardiomyocytes through gap junctions[7,8], propagating received inputs from cardiomyocytes to other electrically coupled cells. In this context, fibroblasts and macrophages are an essential component of normal cardiac conduction, to avoid asynchronous beating and arrhythmias.

Cardiac stromal cells are also responsible for building the proper scaffold of the heart, ensuring correct cardiomyocyte alignment and function. After injury, this 3D structure is fully restored in the hearts of lower vertebrates such as fish and salamanders, which are inherently regenerative: inflammatory responses to damage are rapidly resolved, cardiomyocytes de-differentiate and re-enter the cell cycle to rebuild lost contractile tissue, and transient scar tissue is resorbed[9,10] . The neonatal mammalian heart retains the regenerative potential of the growing embryo, but this capacity is progressively lost after birth[9] . In adult mammalian hearts, repair through scar deposition, although necessary to avoid rupture, leads to adverse remodelling of the ventricular chambers that results in cardiac dysfunction and consequent heart failure.

Why can't adult human hearts heal? The heart contains the only mammalian muscle type that has lost its regenerative potential. In skeletal muscle injury, tissue progenitors (satellite cells) are brought out of quiescence by inflammatory signals, whereupon these tissue progenitors proliferate, differentiate into skeletal muscle cells, and repopulate lost or dysfunctional muscle tissue[11]. Cells of the skeletal muscle stroma concomitantly secrete pro-angiogenic signals to promote revascularization of damaged areas, and orchestrate the replacement of transient interstitial scar tissue with functional muscle. These features are retained during postnatal life and, although tissue reparative capacity generally declines with age, the skeletal muscle is capable of effective regeneration well into maturity of the individual. The proficient regenerative properties of skeletal muscle have spurred an intense search for an elusive "cardiac stem cell" that would be activated in a similar fashion upon myocardial injury. Inherent ontological differences between these muscle types presumably underlie the repeated failure to identify or induce a robust cardiac stem cell population in any vertebrate species.

As an alternative approach, prospects for clinical intervention in human heart disease have been refocused on the dynamic interplay between the immune and cardiac stromal cells, which hold the key to cardiac repair in other organisms, such as zebrafish[12,13] and axolotl[14]. These stromal cell types have critical roles in cardiovascular development and in animals with cardiac regenerative capacity, they participate in launching an anti-inflammatory cascade in response to

injury, promoting a permissive local environment for effective cardiac repair. A compromised immune response, leading to an imbalance in the dynamics of stromal cell-cell communication underlying these critical processes, derails the cardiac repair capacity. The field of regenerative medicine is poised to discover and interpret this cellular crosstalk, which offers new avenues for therapeutic intervention in heart diseases.

This Review discusses the current knowledge on the interplay of cardiac cells in homeostasis and during the different phases of tissue repair, including communication between circulating and resident immune cells, cardiac stromal cells, and cardiomyocytes, with particular focus on the dynamic interactions between components of the innate immune response and cardiac fibroblasts. Mechanistically, cell communication incorporates direct cell-cell contact (such as electromechanical coupling), indirect paracrine signalling (for example, secretion of growth factors and cytokines), and interactions with the extracellular matrix (ECM). All communication modes are involved in maintaining the homeostatic balance and in supporting pathological tissue remodelling. Finally, the Review highlights the potential therapeutic implications of tuning this complex cellular crosstalk to regulate the balance between inflammation and fibrosis to induce a more effective cardiac repair.

[H1] Cardiac injury and repair

Pathological insults in the absence of an external pathogen, such as myocardial infarction (MI), lead to cardiac damage and activate a sterile inflammatory response to extensive cell death and blood vessel damage, causing bleeding and activation of the complement cascade and haemostatic events - all of which lead to the release of pro-inflammatory mediators[5]. Production of reactive oxygen species (ROS) and release of pro-inflammatory cytokines, such as tumour necrosis factor (TNF), IL-1β, and IL-6, activate cell adhesion molecule synthesis in endothelial cells and upregulate the expression of integrins in leukocytes, facilitating the extravasation of immune cells into the injury site. Haematopoietic progenitors migrating from the bone marrow to the spleen give rise to circulating neutrophils and monocytes that are

rapidly recruited to the damaged cardiac tissue through the sensing of chemokine gradients[15] . Furthermore, local cell necrosis induces the release of endogenous damage-associated molecular patterns (DAMPs). Different molecules act as DAMPs, including fragments of the damaged ECM, or intracellular components such as heat shock proteins, ATP, nucleosomes, mitochondrial elements, and alarmins such as high-mobility group box 1 (HMGB1) protein, IL-1α, IL-33, and S100 proteins[16, 17],, which either have a housekeeping, pro-reparative role or initiate inflammation. DAMPs are rapidly released by necrotic cells, including cardiomyocytes and endothelial cells, among others, but are also specifically secreted by active immune cells, fostering the inflammatory response. DAMPS alert the immune system of the tissue damage through activation of pattern-recognition receptors (PRRs) present in immune cells as well as in structural cells such as cardiomyocytes, fibroblasts, and endothelial cells[16,3,4]. PRR activation on fibroblasts induces their proliferation, migration, differentiation into myofibroblasts **[G]** , ECM turnover, and production of fibrotic and inflammatory factors[18, 19-23]. Crucial PRRs involved in the response to myocardial ischaemia are Toll-like receptor (TLR) 2 and TLR4 and the receptor for advanced glycosylation end products (RAGE)[24] . These PRRs recruit and activate PRR-expressing cells of the innate immune system, including neutrophils and dendritic cells, and thus directly or indirectly promote adaptive immunity responses[16].

In the adult mammalian heart, the repair response after injury involves fibrosis. Various stimuli induce different forms of fibrosis, classified as reactive and reparative fibrosis[6]. Reactive fibrosis is not directly associated with cardiomyocyte loss and is due to perivascular collagen deposition in response, for example, to pressure overload or normal ageing[25]. Reparative fibrosis or scarring is accompanied by cardiomyocyte death, and is the result of a highly dynamic and regulated repair process divided into three distinct but overlapping phases: inflammation, proliferation, and maturation[26]. Cardiomyocytes, vascular cells, tissue-resident macrophages, and fibroblasts are all critical players in maintaining cardiac homeostasis and in shaping the crosstalk with immunological factors after myocardial injury. A more nuanced understanding of these processes might help to develop strategies to protect the heart from immune-mediated damage[27]. Numerous innate and adaptive immune cells reside in the heart under homeostatic conditions, including macrophages, dendritic cells, granulocytes, and lymphocytes[28-30]; however,

the cell profile changes radically after cardiac injury (Figure 1).

Neutrophils are the most abundant incoming leukocytes within the first 24h after MI[5]. In a mouse model of MI induced by permanent coronary ligation, neutrophils peak at 1–3 days, and decline by 5–7 days[31]. Neutrophils release high levels of ROS and secrete proteases and pro-inflammatory mediators, which exacerbate local injury and recruit other inflammatory leukocytes. Despite being essential to initiate the acute inflammatory response, neutrophil activation needs to be tightly regulated[31,32]. The short half-life of neutrophils might be an evolutionary adaptation to protect the host from excessive damage[33]. Upon engulfment of pathogens or debris, neutrophils undergo apoptosis and activate 'find me' and 'eat me' signals that promote clearance by macrophages[34]. The engulfment of apoptotic neutrophils changes macrophage phenotypes, inducing the release of anti-inflammatory and reparative cytokines such as transforming growth factor-β1 (TGFβ1), IL-10, and pro-resolving lipid mediators[35], contributing to the resolution of the inflammatory phase.

Although neutrophils have long been considered as detrimental to the MI response, neutrophil depletion in the mouse MI model of permanent coronary ligation leads to heart failure[32]. Neutrophils support the recruitment of pro-inflammatory LY6C^{high} monocytes, which dominate the injury site between day 1 and day 4 after injury, sustaining inflammation and responsible for the removal of debris[36]. In the absence of neutrophils, recruitment of pro-inflammatory monocytes is reduced, and the functions of these monocytes are not supplanted by resident macrophages, which lack phagocytic activity and have a more pro-reparative phenotype. This absence of pro-inflammatory myeloid cells leads to an increase in the number of myofibroblasts and of collagen deposition, with the consequent tissue remodelling leading ultimately to heart failure[15-32.]

Circulating, pro-inflammatory, CCR2^{+}LY6C^{high} monocytes arrive to the damaged area as early as 30 minutes after MI, mostly in response to increased levels of CCL2 in the infarct area [36]. In mice, up to 40% of infiltrating monocytes are generated in the spleen[36]. Egression of monocytes from the spleen is stimulated by circulating angiotensin II[37], regulated by noradrenergic neurons[38], whereas the cholinergic parasympathetic response has an anti-inflammatory

function through vagal nerve stimulation directly on the spleen[39]. The initial wave of pro-inflammatory monocytes is followed by a less intense wave of pro-reparative monocytes (LY6C^{low}CCR2^{-}CX3CX1^{high}), recruited in response to fractalkine, a chemokine that prevails in the infarct area between day 5 and day 16 after MI[26]. Proliferative phase monocytes, macrophages, and endothelial cells coordinate angiogenesis, which provides blood supply to the granulation tissue **[G]** and support myofibroblast proliferation and collagen deposition (Fig. 1).

Why doesn't this flurry of immune activity in response to cardiac damage promote a more successful regenerative outcome? In mammals, arresting monocyte release from the spleen with angiotensin II inhibitors or by splenectomy results in a substantial improvement of cardiac function after MI, underscoring the complex nature of the immune response to cardiac injury[37]. Different studies suggest that the timely resolution of inflammation is more beneficial than complete inhibition of inflammation[40]. The lack of proliferative capacity of adult mammalian cardiomyocytes in response to cardiac damage has been suggested as a critical block in the repair process [41,42]; however, the situation might be more complex. In efficiently regenerating organisms such as salamanders, where resident macrophages support heart regeneration, the injured heart depleted of phagocytic cells cannot regenerate despite normal activation of cardiomyocyte proliferation, which is insufficient to prevent formation of a permanent, highly crosslinked ECM scar[14]. The importance of timely activation of the innate immune response is illustrated by the dramatic difference in cardiac regenerative capacity between zebrafish and the phylogenetically related medaka, in which delayed recruitment of macrophages disrupts neovascularization, neutrophil clearance, and heart regeneration[12]. Taken together, these observations have prompted a refocusing of efforts on a more intensive investigation of the dominant interstitial players deciding the fate of the injured heart.

[H1] Phagocytic macrophages in cardiac repair

Phagocytic macrophages comprise a wide array of innate immune cell subtypes that have diverse roles in the inflammatory response to myocardial damage. Élie Metchnikoff was the first

to appreciate that phagocytosis is more than the simple removal of pathogens and debris. In his original formulation published in 1892, phagocytes represent an evolutionarily conserved cell lineage that performs "identity functions" during embryogenesis as well as in pathological disease. Phagocytosis is also an active defence mechanism and provides restorative and constructive clues [43,44]. In this view, inflammation itself is an ongoing process of self-definition[37], where the very act of subsuming debris is informative and has a crucial role in development, pattern formation, and regeneration[45]. As specialized phagocytes, macrophages reside in all tissues and are involved in tissue growth and remodelling from the earliest stages of development[28,46]. Macrophage depletion impairs the capacity of primitive organisms and young mammals to regenerate, highlighting the critical role of macrophages in tissue repair after injury [14,47].

In the heart, resident macrophages have been extensively characterized as an integral component of organ development. Study of murine models has revealed that the heart contains a large pool of resident macrophages that originate in the yolk sac at very early stages of development, a pool that is maintained into adulthood [48]. The first wave of macrophage progenitor production occurs early in mouse development (embryonic day 7.5), when blood islands are formed in the yolk sac **[G]**. Once embryonic blood circulation has been established, haematopoiesis persists in the fetal liver, and later in the bone marrow, where haematopoiesis remains throughout adult life. The majority of macrophages present in the homeostatic heart are of yolk sac origin[36,46], persisting through embryogenesis into adulthood[49]. These resident macrophages, characterized by a $CCR2^{-}MHC^{low}$ cell surface signature, are found predominantly in the myocardial wall in close association with blood vessels during embryonic heart development, and their depletion leads to malformations of the coronary vasculature, which supplies and drains blood irrigating the heart.

In myocardial injury, $CCR2^{-}$ macrophages promote vessel remodelling through secretion of insulin-like growth factors, which in turn activate angiogenesis[46]. Maintenance of yolk sac-derived tissue macrophages depends on macrophage colony-stimulating factor 1 (CSF1), and genetic ablation of *Csf1* impairs coronary plexus remodelling[46]. Although cardiac macrophages

can be stimulated to proliferate *ex vivo* by co-culture with cardiac interstitial cells, this capacity is lost with CSF1 blockade[9].

Macrophages also control the transient regenerative response in neonatal mammalian hearts [47,49]. Macrophage responses after MI have different magnitude and activation kinetics at postnatal day 1 (P1; regenerative response) and at P14 (pro-fibrotic response)[47]. At P14, the activity of the cell recruitment cytokines CCL2, CCL3, CCL4, and CXCL2 is dampened and angiogenesis and oxidative stress response signals are enrichment compared with P1, whereas P14 macrophages have higher levels of cell presentation molecules and G protein-coupled receptors than P1 macrophages[47]. Interestingly, in adult hearts the majority of the resident macrophages seeded during embryonic development die soon after MI[50]. A similar phenomenon has been described in serosal tissues such as in the peritoneal cavity, referred to as disappearance reaction[51,52]. However, depletion of P1 macrophages by clodronate-mediated phagocyte ablation leads to a significant decrease in cardiac function and increased fibrotic scars in P1 animals after MI[24], underscoring the critical role of macrophages in the regenerative process. Further studies have revealed that the proficient cardiac regenerative response in P1 neonates is due to the persistence of resident embryo-derived macrophages, which induce negligible inflammation and sustain cardiomyocyte proliferation and coronary angiogenesis[49], suggesting that the response of P1 macrophages and other immune cells at early age is fundamentally different to that of P14 cells under the same cytokine stimulus.

The paradigm of M1 (pro-inflammatory) versus M2 (pro-healing) macrophage polarization is based on distinct activation status in response to different stimuli, as seen in T helper cells (Th1 and Th2), where stimuli such as IFNγ, LPS, TNF, and others lead to a pro-inflammatory activation phenotype, whereas IL-4, IL-10, and glucocorticoids induce a pro-healing, immunoregulatory phenotype[53]. This simplified paradigm ignores the source and context of the stimuli, which do not act individually in vivo but are present as a complex cocktail in the tissue milieu. Moreover, macrophages cannot be easily parsed into distinct subset categories, but exist in a continuum with differential cell surface signatures and functions (Table 1). The emerging plasticity of macrophage ontogeny, function, and molecular features has shifted the classical pro-

inflammatory versus anti- inflammatory paradigm towards a network model that incorporates embryological origin and environmental stimuli and timing, as cells are tailored to supply the organ needs[54].

Embryological origin is a defining factor for macrophage identity and specialization: in the brain, replacement of microglia (local macrophages) with bone marrow-derived counterparts yields differential cell signatures[55]. Embryo-derived, tissue macrophages have self-maintenance properties at their respective tissue locations and are resistant to DNA damage from ionizing radiation, as demonstrated for Langerhans cells (skin macrophages), a remarkable property not shared by bone-marrow derived macrophages[56]. Although these studies confirm a fundamental role for immune cell ontogeny in dictating organ response to challenges, local interactions with stromal components also clearly represent a driving force in determining macrophage properties and behaviour, as most organs harbour embryo-derived resident macrophage populations with distinct specialized functions[28,57-61]. Tissue macrophages also conserve differential organ-specific molecular signatures[29,62-65], highlighting the importance of local stromal interactions in the regulation of macrophage identity and function. Whether in the adult heart, the progressive, age-related replacement of embryo-derived cardiac macrophages by bone marrow-derived macrophages[66] limits the potential for repair[67] remains to be demonstrated.

[H1] Dendritic cells in cardiac repair

Dendritic cells are a heterogeneous population of antigen-presenting cells and potent stimulators of the immune response[68]. First identified in the spleen, dendritic cells are found in other lymphoid and non-lymphoid tissues. The low number of dendritic cells in vivo and the overlap of markers and functions with other cell types have made this cell type difficult to study. Dendritic cells can be divided in two main populations: classical or conventional dendritic cells (originated from myeloid precursor cells) and plasmacytoid dendritic cells (originated from lymphoid precursor cells). Classical dendritic cells are grouped together with monocytes and

macrophages as mononuclear phagocytic cells[43]. Classical dendritic cells respond to DAMPs through TLR2 and TLR4 and produce cytokines such as IL-12, IL-23, and TNF . Classical dendritic cells include lymphoid tissue-resident dendritic cells as well as non-lymphoid tissue migratory dendritic cells located at different tissues, such as skin, lung, heart, kidney, liver, and intestine[69], where these cells act as mobile sentinels and upon activation migrate to lymphoid organs where they accumulate to stimulate T cells. Plasmacytoid dendritic cells are similar to plasma cells **[G]**, circulate in the blood, recognize oligodeoxynucleotides via TLR7 and TLR9, and produce IFNα in response to exogenous viruses[70]. Whereas other antigen-presenting cells such as macrophages and B cells can only activate memory T cells, dendritic cells can activate both naïve and memory T cells **[G]**, making plasmacytoid dendritic cells the most potent antigen-presenting cells connecting innate and adaptive immune responses[43,69,70].

In the context of MI, dendritic cells have been reported to accumulate early after injury in the infarct border zone in rats[71] and mice[72], with cell numbers peaking at day 7[73]. Ablation of $CD11c^+$ dendritic cells in mice for 7 days resulted in deteriorated left ventricular function after MI compared with control mice[73]. Dendritic cells also activate both conventional $FOXP3^-CD4^+$ T helper cells and $FOXP3^+CD4^+$ regulatory T (T_{reg}) cells **[G]**, which can prevent tissue-destructive autoimmunity after cardiac injury[74]. A decrease in the number of dendritic cells is associated with cardiac rupture after MI[75], with increased recruitment of pro-inflammatory monocytes that sustain the production of pro-inflammatory cytokines, thereby prolonging the degradation of the ECM and reducing endothelial cell proliferation rates. Although dendritic cell-mediated regulation of monocyte and macrophage homeostasis after MI points to an early beneficial role of dendritic cells in injury repair, these cells might also contribute to excessive fibrosis and later adverse tissue remodelling, drawing attention to the dynamic regulation of fibroblast action during the response to cardiac injury. The role of endogenous dendritic cells in MI-induced cardiac autoreactivity and their effect on heart failure is still under investigation[76].

[H1] Lymphocytes in cardiac repair

A fairly poor adaptive immune cell diversity and responsiveness is a feature of regenerative vertebrates such as fish and salamanders, which might have evolved sophisticated innate immune strategies that reduce the dependency on adaptive immunity for confronting challenges such as infection[77]. By contrast, warm-blooded vertebrates, which have limited cardiac regenerative capacity, have a highly-specialized adaptive immune system with a range of added functions[77]. This difference in the adaptive immune system suggests a potentially adverse role for lymphocytes in cardiac repair.

T cells and B cells are central cellular components of adaptive immunity that arise from lymphoid progenitor cells in the bone marrow. Stimulation of T cells towards either an effector or regulatory phenotype is modulated by dendritic cells, which present self-antigens to T cells to induce a regulatory or tolerogenic phenotype **[G]** under homeostatic conditions or an auto-reactive effector phenotype in response to inflammation.

Both T cells and B cells modulate wound healing and tissue remodelling after myocardial injury[78]. After MI, effector T cells **[G]** are activated in proximal lymph nodes and promptly colonize the damaged heart[78], whereas B cell numbers peak later after the onset of ischaemia[79], producing pro- inflammatory cytokines that reduce cardiac contractility and promote cardiomyocyte apoptosis[80]. Although strategies for therapeutic immune intervention after MI have largely focused on the acute inflammatory phase, the adaptive immune reactivity against cardiac auto-antigens released by tissue damage represents a more insidious process in mammals, which might destroy unaffected cardiac tissue and confound therapeutic attempts at cardiac repair[27]. This ongoing, autoimmune tissue destruction by antigen-specific effector T cells accelerates heart failure by activating fibroblasts into myofibroblasts, which contribute to dysfunctional cardiac remodelling[81] or, by indirect interaction, stimulate innate immune cell-mediated fibrosis[76].

Effector T cells are controlled by the expansion of T_{reg} cells, which protect against adverse ventricular remodelling and sustain cardiac function through inhibition of pro-inflammatory cell infiltration and by direct protection of cardiomyocytes[82]. In zebrafish, T_{reg} cells promote precursor cell proliferation, activating Nrg1 in the heart through Il-10 secretion[13] In mice, T_{reg}

cells probably attenuate chronic cardiac remodelling after MI by suppressing autoreactive T cells **[G]**, because T_{reg} cell ablation induces autoimmunity and cardiac dysfunction[83], whereas removal of effector T cells increases recruitment of pro-inflammatory monocytes and reduces neovascularization and collagen deposition[74].

The beneficial effects of some therapies after MI have been attributed to immunomodulation: current approaches aiming to restore the balance between inflammatory and regulatory immune cell functions include the use of statins, which enhance T_{reg} cell proliferation and activity while inhibiting pro- inflammatory T cell subpopulations[27] Although this transiently impaired pro-inflammatory function renders infants susceptible to infection and prone to allergies, it might also provide an immunological environment permissive of organ regeneration[27]. Notably, the neonate zebrafish has an immature adaptive immune system skewed towards T_{reg} cells[13]. Adult zebrafish fully regenerate the heart after insults such as apical resection, cryoinjury and others.

[H1] Fibroblasts: architects of the heart

As a heterogeneous cell type comprising the connective tissue stroma, fibroblasts are defined as cells of mesenchymal origin, capable of secreting “fibers” such as collagen and other ECM components to maintain tissue integrity[84]. Fibroblasts include resident mesenchymal cells, medullary fibrocytes, myofibroblasts, chordal fibroblasts, and valvular interstitial cells. Often cited as a principal non-myocyte cell type, fibroblasts represent only ≤25% of the non-myocyte cells in the heart, depending on the markers used to identify them[5,58]. High-resolution electron microscopy shows that cardiac fibroblasts, interspersed in the collagen network[85], extend long filipodia and closely associate with cardiomyocytes and other cells of the interstitium such as endothelial cells[86,87]. Along with resident macrophages, cardiac fibroblasts act as insulator cells that connect with cardiomyocytes of the conduction system to maintain regulated rhythmicity[7,88,89]. In the injury context, cardiac fibroblasts respond to immunological stimuli and signal to cardiomyocytes[8], a crucial role in the orchestration of the healing process. Fibroblasts themselves have broad immune-regulatory properties, interpreting inflammatory factors to

signal surrounding stromal cells and initiate production of ECM components for tissue reconstruction[20].

Cardiac fibroblasts originate mainly from two layers of the embryo, endocardial (the endothelial layer lining the heart chambers) and epicardial (an epithelial layer covering the external surface of the chambers) [90-93] (Fig. 2). Cells in both layers undergo epithelial-to-mesenchymal transition during heart morphogenesis. Mesenchymal cells delaminated from the endocardial layer give rise to fibroblasts of the valvular compartment, as well as to a small fraction of interstitial fibroblasts within the muscle walls. Epicardial-derived cells give rise to the majority of fibroblasts of the muscle interstitium (>80%) in atrial and ventricular chambers. How immune cells communicate with cardiac fibroblasts in the embryo remains to be determined, but considering what is known about the adult heart, interstitial cell–cell communication is probably of vital importance to promote a healthy environment for cardiac formation and growth.

ECM deposition is essential during embryonic development for proper morphogenesis[94] not only in shaping the 3D architecture of the organ, but also as a source of growth signals supporting survival and proliferation. Embryonic fibroblasts secrete high levels of fibronectin, collagen, and heparin-binding EGF-like growth factor (HBEGF), which regulate cardiomyocyte mitotic activity (hyperplasia) through β1 integrin signalling[95]. Soon after birth, the neonatal heart faces a substantial increase in systolic pressure and responds by increasing the thickness and tensile strength of the ventricular wall. These changes are achieved through a doubling in fibroblast number and an active remodelling of ECM components during the first week after birth[96]. Interestingly, this period is the time window during which the mammalian heart retains the capacity to regenerate[9]. The percentage of fibroblasts in the neonatal heart is about 41% of the total interstitial cells, as opposed to the ≤25% in the adult heart, suggesting that high numbers of cardiac fibroblasts are important to support cardiomyocyte hyperplasia[97].

In contrast to systemic immune cells that are recruited by cardiac damage, production of adult cardiac fibroblasts is endogenous and independent of any damage stimulus. Fibroblasts are not formed *de novo* (such as by endothelial-to-mesenchymal transition) or recruited from

circulation. but are rather formed through proliferation of pre-existing endogenous progenitors[90-92,98,99].

In the adult heart, cardiac fibroblasts are the main cell type responsible for ECM deposition, expressing high levels of IL-6 and supporting cardiomyocyte hypertrophy[95]. Impaired cardiac fibroblast formation in mice lacking a defining transcription factor TCF21 results in decreased expression of collagens compared with wild-type mice [100]. After apical resection, regeneration occurs in neonatal mice within 21 days, thanks to the active proliferation of cardiomyocytes, which persist until day 7[9]. However different degrees of scarring has been observed, based on the extent and time of resection[101] such that resected P2 hearts have been shown to be more prone to develop fibrosis than P1 hearts[102]. Global transcriptome comparison between the two stages showed differential expression of ECM and cytoskeletal components, and reducing stiffness of the cardiac ECM at P2 via chemical intervention promoted healing, pointing to the microenvironment as a critical component of the regenerative response in neonate mice[102]. Fibroblasts are not the only producers of ECM: macrophages, endocardial cells, and epicardial cells can secrete a broad range of ECM components (reviewed previously[103]; Table 1). Indeed, the proteoglycan agrin, which has been shown to promote heart regeneration and cardiomyocyte proliferation mediated by release of the Hippo effector YAP[104,105], is largely produced by endothelial cells.

The maintenance of tissue architecture through matrix deposition and remodelling in the adult heart is still poorly understood, posing a major challenge for regenerative medicine. Positional memory in fibroblasts probably involves *HOX* genes, which encode transcription factors specifying embryonic positional identity in cells and guiding tissue differentiation. Fibroblasts preserve elements of embryonic *HOX* expression patterns in the adult, where distinct patterns of *HOX* genes define position of adult fibroblasts along developmental axes[1,2]. In the highly regenerative axolotl, the *HOX* code patterns muscle-forming cells during regeneration, regulated by signals from the connective tissue, which provides further guidance for proper antero-posterior patterning of the newly formed limb[3]. Although transcriptional memory of *Hox* gene expression has yet to be documented in the stromal cells of the heart, adult cardiac

fibroblasts retain the cardiogenic transcriptional profile essential for embryonic cardiomyocyte formation[106]. These genes are also implicated in congenital heart disease; for example, conditional deletion of the gene encoding the cardiogenic transcription factor TBX20 in cardiac fibroblasts leads to valvular and septal malformations, as well as hypoplasticity of myocardial ventricular chambers[106].

***[H2] Fibroblasts, pericytes, and mesenchymal cells — a matter of definition*.** The lack of a cell-specific marker has been a limiting factor in the isolation and study of fibroblasts, which are classically isolated on the basis of their capacity to adhere to plastic, presenting a classic spindle-shape morphology in vitro. None of the proposed fibroblast markers uniquely capture the whole fibroblast population[93,107,108], which has been studied as an amorphous group of ECM-producing cells, as passive bystanders in the working myocardium. A confounding factor is that fibroblasts, like other cells in the interstitium, retain broad plasticity, and can change gene expression profile and phenotype depending on the signals in the surrounding environment. Genetic lineage tracing of the bHLH transcription factor TCF21 has shown that the activation response of fibroblasts varies for different stimuli; isoproterenol induces a strong endocardial perivascular response, transaortic banding causes stronger activation at the basal epicardial wall of the left ventricle, and MI leads to a very defined deposition of cells in the apical infarcted area of the left ventricle[109].

This cell plasticity is particularly evident in response to an injury stimulus, when myofibroblasts secrete type 1 collagen to strengthen the infarct area and protect from rupture, followed by ECM remodelling and deposition, formation of the scar, and in some conditions, also formation of adipogenic[110-112] or condrogenic deposits[113], both undesirable features for correct heart function. Interestingly, only a subfraction of activated fibroblasts express the myofibroblast marker smooth muscle actin (ACTA2) [114], raising the question of whether ACTA2^{-} fibroblasts comprise a subgroup of cells with different function.

Given the shared expression of surface markers, transcription factors[106], and functional

properties[115], it could be argued that fibroblasts, cardiac mesenchymal stromal cells (MSCs), and pericytes probably are different presentations of the same cell type that has adapted to perform specialized functions required by their microenvironment[116-120]. Criteria set by the International Society for Cellular Therapy define MSCs as plastic-adherent cells that express the surface antigens CD105, CD73, and CD90, lack expression of the haematopoietic markers CD45, CD34, CD14 or CD11b, CD19, and HLA-DR, and can differentiate at least into osteogenic, chondrogenic, and adipogenic lineages[121]. All these features are also shared by fibroblasts[117]. The potential for MSCs to self-renew and reconstitute all the components of the stroma has been formally determined *in vivo* solely for MSCs derived from the bone marrow[122], and in the absence of compelling evidence documenting MSC differentiation into cardiomyocytes, the existence of a true mesenchymal stem cell in the heart remains speculative[123].

Pericytes, also referred to as Rouget cells or mural cells, are specialized fibroblast-like cells with cytoplasmic processes that envelop endothelial cells in the microvasculature providing structural integrity[124]. In response to injury, pericytes secrete multiple paracrine factors[125] and interact with immune and inflammatory cells, supporting the immunosurveillance and effector functions of extravasated neutrophils and macrophages. The contribution of pericytes to cardiac regeneration derives from their role as mediators of tissue growth signals during development. In therapeutic settings, injected pericytes reduce post-MI scar formation, cardiomyocyte apoptosis, and interstitial fibrosis, recruiting monocytes by secreting growth factors, microRNAs, and chemokines[126], promoting angiogenesis, and inhibiting chronic inflammation[127]. As with other cardiac stromal components, pericytes have contrasting roles depending on context: in acute or chronic inflammation accompanying severe cardiac damage or disease, pericytes are diverted from a pro-regenerative to a pro-fibrotic state**,** generating scar-producing myofibroblasts [128,129] . Single-cell transcriptomic identification of cells resembling a fibrocyte population expressing canonical genes corresponding to both fibroblasts and macrophages and/or leukocytes[30] suggests that blurring of classical interstitial cell identities is likely to be an emerging feature of myocardial cell characterization.

[H1] Conversations in the cardiac interstitium

The dynamic interplay between fibroblasts and the surrounding stroma drives multiple outcomes in the changing cellular landscape of the heart during cardiac inflammation and tissue repair. An extensive inflammatory phase with prolonged protease activity can lead to cardiac rupture or dilation[130,131]. Conversely, excessive scar deposition leads to stiffer ventricles and diastolic dysfunction[6,22]. Increased appreciation for the dense intercellular communication network between diverse cardiac cell types[132] has focused attention on the changing phenotypes of interstitial cells that orchestrate the onset and resolution or maladaptive features of tissue repair.

Often defined as sentinel cells[133], fibroblasts and pericytes are in close contact with cardiomyocytes and other interstitial cells through cell junctions and ion channels, and can promptly perceive mechanical, electrical, and chemical changes in the environment and transit the signals throughout the myocardium by direct physical interaction or secretion of chemokines[133,134]. In this sense, fibroblasts and related cell types behave like a communication hub orchestrating homeostasis and the response to stress. Paracrine signalling from stromal cells has an important role in the cardiac communication network, through direct secretion of factors or through different types of specialized extracellular vesicles, such as exosomes and microvesicles, which deliver cargos of proteins, lipids, mRNA, and microRNA to proximal and distal cells[135-137]. Although crosstalk within the myocardium is widespread, fibroblasts are the most trophic cardiac cell population, secreting ligands for which cognate receptors are detected within cardiac non-myocyte populations[11].

Upon myocardial damage, intracellular communication shifts dramatically. In response to ROS and pro-inflammatory cytokines, fibroblasts are activated to a matrix-degrading phenotype and secrete a cocktail of pro-inflammatory interleukins and chemokines[138,139], suggesting an active contribution to sustaining the early inflammatory phase[140,141]. Human primary cardiac fibroblasts obtained from endomyocardial biopsies of patients with heart failure and dilated cardiomyopathy exposed to mechanical stress increase the production of ECM components, pro-inflammatory chemokines, and factors supporting monocyte recruitment[142]. Fibroblasts are

also involved in phagocytosis of apoptotic cells[143], collagen[144], and neutrophils[145], a feature normally attributed to macrophages[146]. As in skeletal muscle injury[147], the prompt removal of debris from damaged cardiac tissue is orchestrated by fibro-adipo progenitor cells that express PDGFRα[148], a receptor also essential for the generation of cardiac fibroblasts from the epicardium during embryonic development[93,149]. During the inflammatory phase, angiostatic factors such as CXCL10 inhibit angiogenesis while a fibrin-based temporary matrix is deposited and the myocardium is cleared of cell debris[150].

The transition to a pro-reparative, proliferative phase several days after cardiac injury is marked by repression of pro-inflammatory signals[151] and activation of factors that stimulate formation of a granulation tissue, differentiation of fibroblasts into myofibroblasts and their proliferation (Fig. 1). This phase is marked by a peak in proliferation of fibroblasts expressing TCF21, type I collagen, or PDGFRα[109]. The signals that induce termination of the inflammatory phase are not yet fully understood, but the cross-talk between monocytes and/or macrophages and fibroblasts is likely to have a central role in containing the inflammatory response[152-154]. TGFβ is one of the main factors responsible for the conversion of fibroblasts to myofibroblasts and induction of ECM synthesis, because deletion of the downstream TGFβ effector SMAD3 is reported to significantly reduce collagen deposition in the infarcted heart[155]. During this phase, myofibroblast migration and proliferation is driven by platelet derived growth factors, fibroblast growth factors, angiotensin II, and the mast-cell derived proteases chymase and tryptase[26] while VEGF and CXCL12 regulate neoangiogenesis, supporting the formation of a granulation tissue[40]. Activated epicardium is a central source of these angiogenic factors[156]. At this point, TGFβ inhibits the angiostatic factor CXCL10 together with other pro-inflammatory cytokines and chemokines, allowing pro-reparative factors to exert their functions[150].

Two weeks after cardiac injury, maturation signals support ECM deposition and termination of angiogenesis, leaving a mature scar with crosslinked collagen fibres. The cardiac scar has a proactive role in preventing cardiac rupture, preserving ventricular integrity, and transmitting electrical signals. The scar is not acellular, but rich in fibroblasts, endothelial cells, and myofibroblasts, which help to retain cardiac contractility. Although the connective tissue can act

as an electrical insulator, scar-forming fibroblasts can be highly conductive and arrhythmogenic for excitable cardiomyocytes, causing external foci of electrical re-entry[157].

A more refined understanding of the changing intercellular signalling that mediates the timely transition of tissue repair phases in scenarios of functional cardiac regeneration will provide clues to promote the formation of transient scar tissue, which can be resolved to allow improved heart remodelling. Molecular profiling of the heterogeneity of the interstitium at a single-cell level will be fundamental for uncovering the complex network of interactions within the heart under homeostatic conditions, as well as the dynamic evolution of the different cell types in response to cardiac injury[30].

[H1] Clinical prospects

How does an increased appreciation for the critical role of interstitial cells in heart homeostasis and disease translate into the clinic? Current pharmacological treatments in the acute phase after MI are systemic and mainly aimed at vasodilatation and haemodynamic unloading[158]. Acute myocardial reperfusion also confers well-established benefits to local and global ventricular function in the long term. In chronic heart failure, neurohormonal blockade through pharmacological interventions, such as use of β-blockers, angiotensin-converting enzyme inhibitors, and angiotensin II-receptor blockers, is employed to prevent left ventricular remodelling and reduce mortality after MI. These therapies have systemic actions, owing to the shared molecular signatures among resident cells in the myocardium and in the rest of the body, and affect various cardiac cell types in different ways. More targeted and personalized therapies will require a deeper understanding of the complex interactions among resident cardiac cells and systemic signals, and how these signals change and evolve with time after injury.

As emphasized in this Review, interstitial cells have crucial immunological functions and are active players in shaping immune responses. Systemic immunosuppressive therapies impair healing; therefore, current clinical strategies increasingly focus on modulating cardiovascular function rather than targeting inflammation or fibrosis. Many common drugs such as statins and

other lipid-lowering drugs have secondary anti-fibrotic and anti-inflammatory actions[20,84], as well as other effects such as increasing the proliferation and activity of T_{reg} cells while inhibiting pro-inflammatory T cell subpopulations[27] — all features that might underlie the beneficial effects of these drugs. Increasing evidence of the engagement of the immune system in cardiac repair mechanisms underscores the benefits of therapeutic approaches that foster the regenerative aspects of the immune and fibrotic responses, rather than simply suppressing these processes.

[H2] ***Aptamers***. Prospective candidates for novel therapeutic approaches that target the cardiac interstitium include aptamers — short, single-stranded oligonucleotides (RNA or DNA) [159] with high specificity for multiple targets including inorganic molecules, protein complexes, or even entire cells (Fig. 3) Over the past decade, aptamers have been used in several experimental cardiovascular applications, including amelioration of cardiotoxicity, biomarker discovery, and improvement of cardiac muscle contractility[160-162]. Other examples include osteopontin RNA aptamers that reduced cardiac hypertrophy and fibrosis in a mouse model of heart failure induced by pressure overload[163] and aptamers designed to neutralize G-coupled receptor autoantibodies for potential treatment of cardiomyopathies[164] or to neutralize β1-adrenergic receptor autoantibodies as a potential therapeutic strategy in hypertension[165]. Clinical challenges of aptamers-based strategies to be addressed include duration of action and rapid degradation rates, excretion by renal filtration, interaction with nonspecific targets, and automation for mass production[159]. Optimal route of delivery, efficacy, and adverse effect of chronic aptamer administration also remain to be determined[166], however, aptamer-based antithrombotic treatments[167-168] have already moved into clinical trials in patients undergoing cardiovascular interventions[169-173].

[H2] Biomaterials. Application of biomaterials in the cardiovascular field include the generation of decellularized hearts for transplantation and matrices and patches for implantation after MI, most of which still require fine-tuning and proper pre-clinical testing[174]. (Fig. 3). Biomaterials for myocardial repair must be flexible, elastic, and compatible with cardiac cell viability. These biomaterials include functional cardiac patches for implantation onto damaged tissue and

injectable biomaterials to reduce pathological cardiac remodelling[175]. Injectable materials can also contain healing factors or varied cell types to aid in the regeneration process. Natural scaffolding materials such as alginates[176,177], fibrin[178,179], hydrogels, and collagen[180-182] are favoured for tissue engineering owing to their biocompatibility. However, synthetic polyesters such as PCLA (polymer of ε-caprolactone-co-L-lactide)[183], pHEMA-co-MAA (poly(2-hydroxyethyl methacrylate-co-methacrylic acid)[184], PCL (polycaprolactone)[185], and PLA-PLGA (poly-L-lactic, polylactic glycolic acids)[186], or elastomers such PGS (poly-glycerol-sebacate) [187], can be also used owing to their capacity to accommodate to cardiac mechanical activity.

Conservation of cardiac interstitial architecture is the driving objective behind therapeutic investigation of decellularized hearts[188-190], which support remarkable reconstitution of migrating cell types to their original niche, and are capable of responding to electrical stimulation. Before experimental recellularized hearts can be used in the clinic, important hurdles must be overcome, such as the risk of thrombosis in the coronary arteries and the response of host inflammatory cells[191]. Optimizing the sources, types, and maturation of repopulating cells to achieve adult organ function, and reducing the immunogenicity of the transplant will be additional challenges to the clinical application of this promising avenue.

[H2] Biologic therapies. Biologic therapies **[G]** , fostered by advances in recombinant DNA technologies over the past 4 decades, have great potential for the modulation of the cardiac interstitium for cardiac repair and regeneration. These biologic agents include monoclonal antibodies, such as anti-TNF antibodies (Etanercept and Infliximab), anti-CD20 antibodies (Rituximab), receptor and enzyme modulators such as the IL-6 receptor antagonist, Tocilizumab, and the antagonist of the IL-1 type I receptor, Anakira[192], all of which have been tested in clinical trials with varying results[193,194]. Biologic agents targeting chemokines, TLRs, the general removal of autoantibodies, and the TGFβ co-receptor endoglin[193,195-197] are other promising avenues. (Fig. 3). However, most biologic agents have a broad spectrum of action, such that timing and dosing become the main options for modulating their activity.

[H2] MicroRNAs. In contrast to biologic agents, microRNAs are modulators of gene expression that can target selected mRNAs, therefore tempering complex biological processes[198]. Cardiac-

specific microRNAs such as miR-1, miR-133a, miR208a/b, and miR-499 are abundantly expressed and have demonstrated effects in cardiac development and regeneration, heart failure, stroke, and atherosclerosis, where the expression of these microRNAs is altered[199]. (Fig. 3). Although their diagnostic value as biomarkers is still to be determined[200], microRNAs are promising candidates for therapeutic strategies to influence the cardiac microenvironment.

[H2] Nanoparticles and exosomes. Delivery of therapeutic biologic agents by nanoparticles and exosomes is an emerging area of cardiovascular translational research. Engineered nanoparticles (with a diameter in the nm range) have unique physical and chemical properties, such as large surface area to mass ratio, electrothermal conduction, reactive surface groups, and diverse composition[201], and can be modulated for composition, size, and concentration to modulate their kinetics. Nanoparticles protect the therapeutic cargo from diffusion or degradation, and afford sustained delivery of growth factors, enzymes, small molecules, and other factors to the injured heart[202] through intramyocardial or intravenous injections (Fig. 3). Nanoparticle-mediated delivery of insulin-like growth factor 1[203], p38, or NADPH oxidase siRNA[202] have all shown beneficial effects in mouse models of MI. Tissue-targeted accumulation of sufficient therapeutic cargo using systemic nanoparticle injection is challenging, but micelles and liposomes have been effective for delivering prostaglandin E1, ATP, coenzyme Q10, angiotensin I, and VEGF to the infarcted heart with the use of intravenous injection[204-208].

Exosomes have generated considerable excitement as vehicles for cardiovascular therapeutic delivery. As small, naturally occurring extracellular vesicles, with inside-out plasma membranes produced through the exocytosis pathway[209], exosomes carry adhesion proteins or lipid moieties on their surface, but do not generate an immune response. In the heart, exosomes are produced and released by multiple cell types including cardiomyocytes and fibroblasts, and carry small cargo molecules, such as microRNAs, for intercellular communication, which are secreted in homeostatic and pathological conditions[136,210]. Exosomes secreted by cardiac fibroblasts can mediate cardiomyocyte hypertrophy in a paracrine manner through the action of miR-21[211], and other exosomes have been identified in other disease settings including MI[210]. (Fig. 3). Because of their ready availability in the blood stream, correlation with pathologies, and

cell-specific membrane and cargo composition, exosomes are highly attractive as biomarkers for cardiovascular disease[126]. Challenges in characterizing the complex composition of endogenous exosomes and the lack of adverse effects information and methods to isolate or produce exosomes in sufficient quantities, all could be overcome by biosynthetic mimetics, which could be designed for defined composition and targeting.

[H1] Conclusions

Understanding the development and functions of the different cellular protagonists – and perhaps more importantly the critical timing of their potential crosstalk in cardiac homeostasis and disease – will help to design new regenerative medical strategies for treatment of cardiac diseases] The identification of new clinical biomarkers such as exosomes, secreted proteins, aptamers, and miRNAs will facilitate intervention on the dynamic interplay of cardiac cell types to improve cardiac repair and help to prevent the development of ischaemic heart failure. Promising new avenues for clinical intervention are emerging, but we are only at the beginning of an exciting era in cardiovascular therapy, where the focus shifts from pharmacological modulation of systemic pathways to the deployment of more specific targeting of endogenous immune and stromal cell–cell communication and response, tapping into the inborn intelligence of the system.

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Key points

- Cardiac interstitial cells have critical roles in cardiovascular development and in maintaining the correct 3D scaffold of the heart in homeostasis.
- The dynamic interplay between cardiac stromal cells and circulatory immune cells can either support tissue regrowth in regenerative organisms or fail to resolve inflammation and produce fibrotic scar tissue.
- The response to myocardial injury proceeds in three overlapping phases: inflammation, proliferation, and maturation; the dynamics of inflammatory and proliferative phases influence the reparative outcome.
- Understanding the development and functions of different cardiac cellular components, and the critical timing of their potential crosstalk in tissue homeostasis and disease, will help to design new regenerative therapeutic strategies
- Promising new therapeutic strategies are emerging, with a shifting focus from pharmacological modulation of systemic pathways and stem cell-mediated therapies to more specific targeting of the endogenous immune–stromal cell interplay

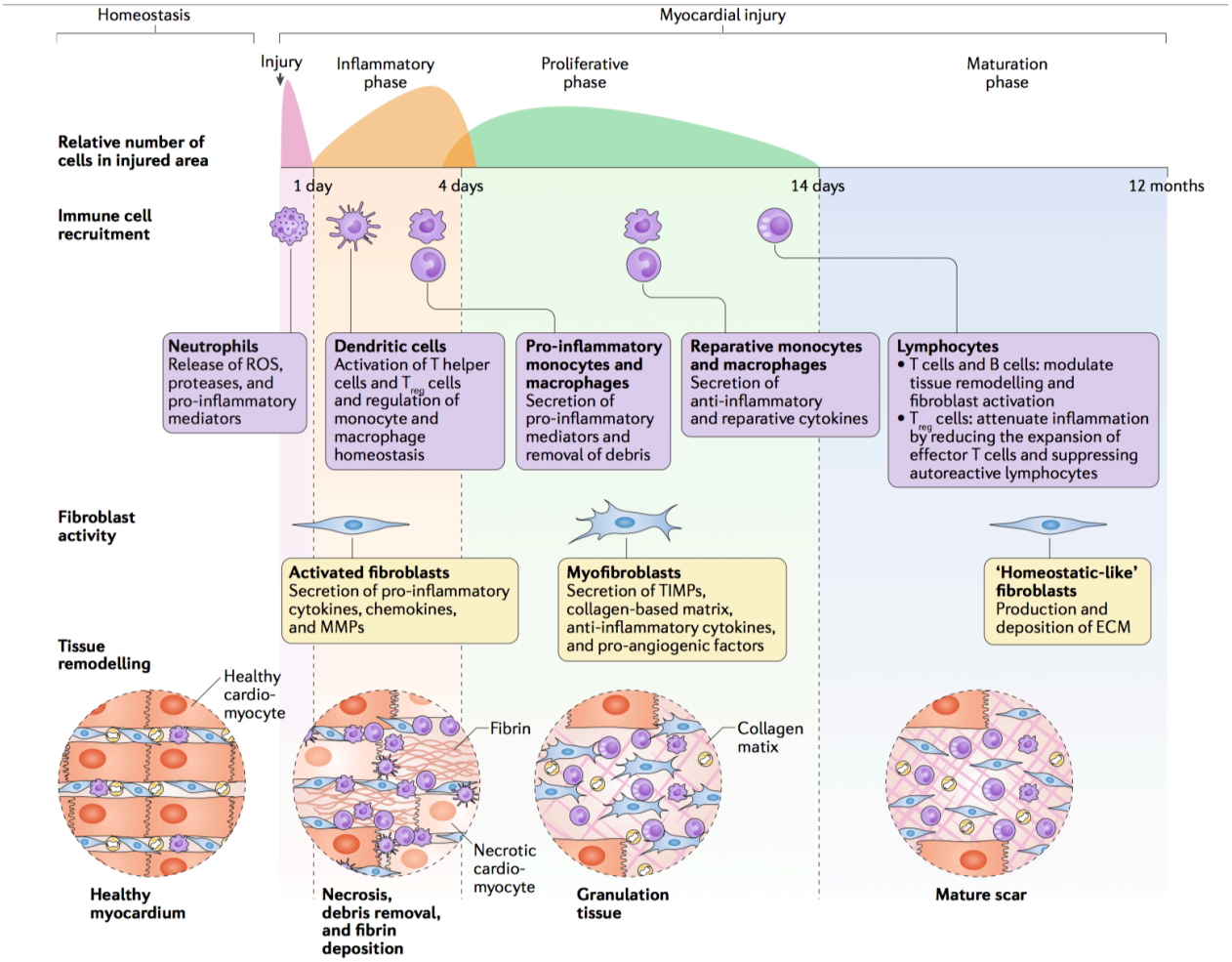

Figure 1. Immune cell and fibroblast functions after myocardial injury. A pathological insult such as myocardial infarction leads to ischaemic damage, sterile inflammation, and cardiomyocyte death. The repair response after cardiac injury can be subdivided into three overlapping phases: inflammatory, proliferative, and healing or maturation. In the early inflammatory phase, cardiomyocyte death leads to the release of damage-associated molecular patterns (DAMPs) and the activation of pattern-recognition receptors (PRRs) in immune cells, cardiomyocytes, fibroblasts, and endothelial cells, neutrophil infiltration, and recruitment of systemic monocytes and resident macrophages all of which promote clearance of debris and the deposit of a temporary fibrin matrix to replace dead cells. In the subsequent proliferative phase, inflammation is contained by a pro-healing subset of monocytes and macrophages, accompanied by recruitment of lymphocytes, angiogenesis, and myofibroblast differentiation, and a collagen-based matrix replaces the initial fibrin deposition (granulation tissue). The last,

healing phase involves the formation of a mature scar, mostly devoid of cardiomyocytes. In this stage, myofibroblast activation recedes. A mature, dense, collagen network containing fibroblasts, immune cells, and microvasculature are part of the mature scar tissue.

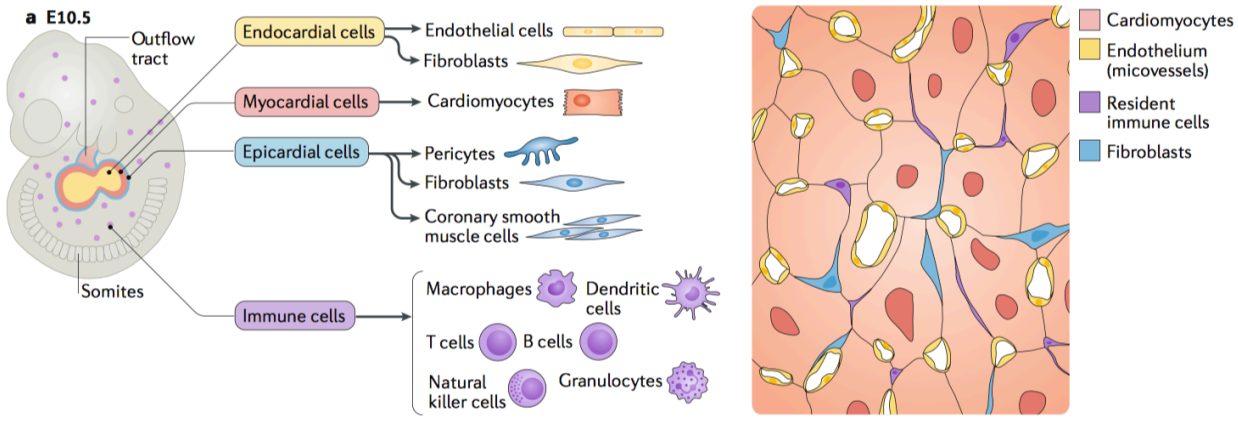

Figure 2. Ontogeny of the cardiac interstitium. A. During early development, immune cells (purple) are produced in the yolk sac and infiltrate various tissues of the embryo, including the heart. At embryonic day 10.5 (E10.5) the heart is composed of three tissue layers; the endocardium (yellow), the epicardium (blue), and the myocardium (red). The endocardium is formed by endothelial cells that undergo epithelial-to-mesenchymal transition from E9.5 to form valvular tissue and a subset of interstitial fibroblasts. The epicardium envelops the external surface of the heart and gives rise to most interstitial fibroblasts, smooth muscle cells, pericytes, and a small proportion of coronary endothelium. The myocardium is formed by cardiomyocytes, the beating muscle unit. **B.** Structural organization of cardiac muscle depicting the interaction between cardiomyocytes and interstitial fibroblasts, immune cells, and luminal endothelial cells.

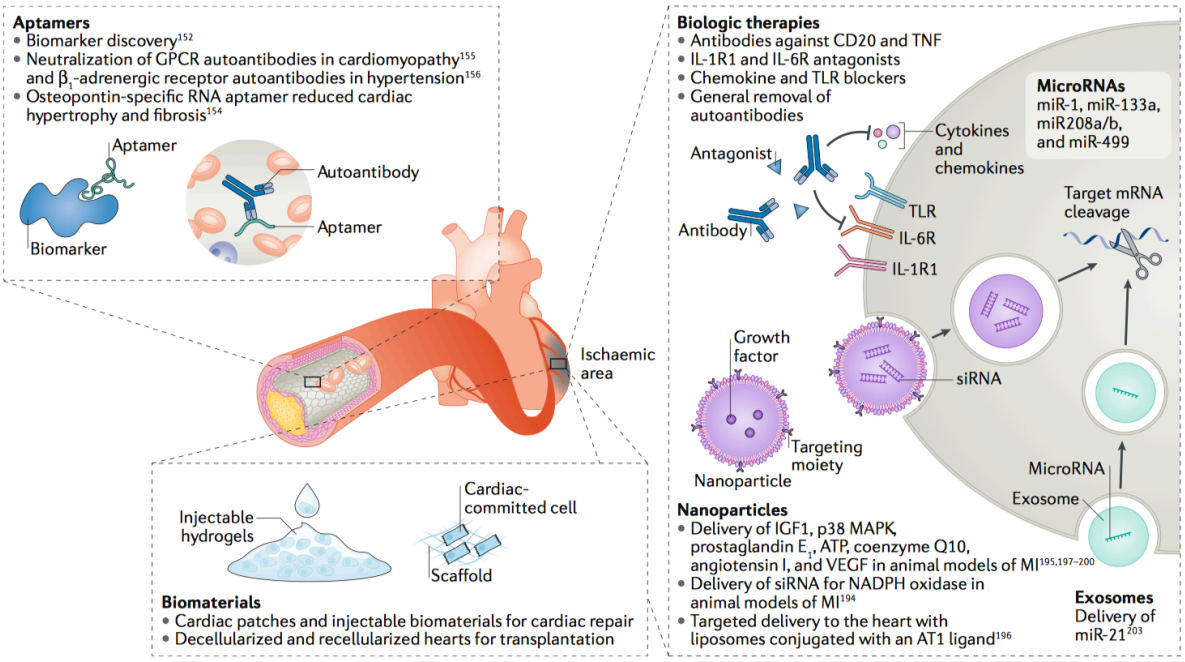

Figure 3. Potential therapeutic strategies targeting the cardiac interstitium. An overview of potential therapeutic strategies to target cardiac fibrosis and the inflammatory response to myocardial infarction (MI), which include the use of aptamers, biomaterials, and biologic therapies such as microRNAs, antibodies, and growth factors, is shown. AT1, type 1 angiotensin II receptor; GPCR, G protein-coupled receptor; IGF1, insulin-like growth factor 1; IL-1R1, IL-1 receptor type 1; IL-6R, IL-6 receptor; p38 MAPK, p38 mitogen-activated protein kinase; siRNA, small interfering RNA; TLR, Toll-like receptor; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

Table 1. Current markers for major cardiac resident cell populations of the mouse heart.

Cell type	Markers (embryonic and/or adult)	Embryological origin
Cardiomyocytes	• *Myh6* and *Myh7*[97] • *Acta1*, *Actc1*, *Atp2a2*, *Nppa*, *Ryr2*, *Tnnc1*, *Tnni*, *Tnnt2*, and *Tpm*[212] • *Gata4*, *Foxh1*, *Hand1*, *Hand2*, *Isl1*, *Mef2a*, *Mef2c*, *Mesp1*, *Nkx2-5*, *Tbx1*, *Tbx3*, *Tbx5*, *Tbx20*, *Srf*, and other transcription factors[97,213] • *Tbx18* and *Wt1* (septal)[214] *Tbx18* and *Wt1* (septal)[214] • *Myoz2* (subepicardial)[212]	Pre-cardiac mesoderm, myocardial progenitors[215]Pre-cardiac mesoderm, myocardial progenitors[215]
Cardiac stem cells	• *Kit*[216] • *Sca1* and Hoechst 33342 chromatin dye[217]	Undefined and/or epicardial[84,222]

	• *Pdgfra*[218] • BCRP1[219]BCRP1[219] • *Thy1* (also known as CD90), *Pdgfra*, *Sca1*, and *Wt1*[220] • *Isl1*[221] • *Isl1*, *Nkx2-5*, *Tbx18*, and *Wt1*[222] *Isl1*, *Nkx2-5*, *Tbx18*, and *Wt1*[222]	
Fibroblasts	• *Col1a1*, *Col1A2*, *Ddr2*, *Fsp1*, *Pdgfra*, *Postn*, *Tcf21*, *Thy1* (also known as CD90), and *Vim* [93,149] • *Acta2* (myofibroblasts)[149]*Acta2* (myofibroblasts)[149] • *Flna*[223] • *Klf5*[224] • *Gata4*, *Gata5*, *Gata6*, *Hand2*, *Sca1*, *Tbx2*, *Tbx5*, *Tbx20*, *Tcf21*, and *Wt1*[106] • *Abi3bp*, *Adamts5*, *Clec4d*, *Csf3r*, *Dkk3*, *Dpep1*, *Entpd2*, *Frzb*, *Gstm5*, *Hcar2*, *Hdc*, *Hp*, *Igfbp6*, *Lamb1*, *Lamc1*, *Lmnb1*, *Mdk*, *Medag*, *Meox1*, *Ms4a4d*, *Pcsk6*, *Prg4*, *Retnlg, S100a8*, *S100a9*, *Slpi*, and *Wif1*[30a] • *Col3a1*, *Fbln2*, *Fstl1*, *Gsn*, *Mmp2*, and *Sparc*[212,a]	Epicardial and endocardial[84,92,103]
Pericytes	• *Acta2*, *Anpep* (also known as CD13), *Col4*, *Des*, *Ng2*, and *Pdgfrb*[225]*Acta2*, *Anpep* (also known as CD13), *Col4*, *Des*, *Ng2*, and *Pdgfrb*[225] • *Abcc9*, *Cog7*, *Col1a1*, *Colec11*, *Gnb4*, *Heyl*, *Kcnj8*, *Myo1b*, *P2ry14*, *Steap4*, and *Vtn*[30,a]	Undefined or epicardial[225] Undefined or epicardial[225]
Endothelial cells	• *Tie1* and *Tie2*[226] • *Pecam1*[227] • *Sca1*[228] • *Flk1* and *Flt1*[229] • CD106, CD144, and vWF[230] • *Wt1*[231] • *Cdh5*[232] • *Ednrb*, *Emcn*, and *Epas*[212] • *Egfl7*, *Gpihbp1*, *Ly6c1*, *Mgll*, *Rgcca*, and *Slc9a3r2*[30,a]	Pre-cardiac mesoderm, endocardial[215] and/or epicardial[233]Pre-cardiac mesoderm, endocardial[215] and/or epicardial[233]
Lymphatic endothelial cells	• *Pecam1* • *Lyve1*[234]*Lyve1*[234]	Venous vasculature[235]
Smooth muscle cells	• Acta2 and Tagln[236]Acta2 and Tagln[236] • Pdgfrb[237] • Myh11[238] • Flna[223] • Cola1, Des, Lmod1, Mustn1, Mylk, Nrip2,Pcp4l1, Pln, and Sncg[30,a]	Undefined and/or epicardial[237]
Epicardial cells	• Pan-cadherin[220] • Tcf21[100] • Tbx18 and Wt1[214]Tbx18 and Wt1[214] • Aldh1a2, Anxa8, Bnc1, C2/C3 complement, Chi3l1, Cyp2s1, Dmkn, Efemp1, Gpm6a, Igfbp6, Ildr2, Krt8,	Pro-epicardial organ[240]Pro-epicardial organ[240]

	Krt19, Lrrn4, Mpzl2,Muc16, Nkain4, Prr15, Saa3, Slc26a3, Slc39a8, Slc9a3r1, Upk1b, and Upk3b[239]Aldh1a2, Anxa8, Bnc1, C2/C3 complement, Chi3l1, Cyp2s1, Dmkn, Efemp1, Gpm6a, Igfbp6, Ildr2, Krt8, Krt19, Lrrn4, Mpzl2,Muc16, Nkain4, Prr15, Saa3, Slc26a3, Slc39a8, Slc9a3r1, Upk1b, and Upk3b[239]	
Monocytes and macrophages	• Adgre1, CX3CR1, CCR2, CD11b (also known as ITGAM) , CD11c, CD45, CD64, CD206, LY6C, MHC II, MERTK, and MRC1[28] • CD68, CD163, Csfr1, Emr1, Lgals3, and Lyz1[212] • Ccl5, Col1a1, Ctsw, Dab2, Gsmb, Gzma, Klrb1c1, Klrd1, Klre1, Klrk1, Mgl2, Ncr1, and Nkg7[30,a]	Yolk sac, embryonic liver (homeostasis), bone marrow (injury)
Dendritic cells	• CD11b, CD11c, CD45, CD103, and Zbtb46[28] • CD209a[241] • Ccr2, Ear3, H2afy, Ifitm6, Lgals3, Naaa, Napsa, Plac8, Plbd1, and Rnase6[30,a]	Undefined or myeloid-derived (nonlymphoid tissue-resident and/or migratory classical dendritic cells)
Lymphocytes	• B cells: CD20 and Ms4a1[242]; Bank1, Ccr7, CD55, CD79a, CD79b, Col1a1, Fcmr, Ly6d, H2-Dmb2, H2-Ob, and Ms4a1B cells: CD20 and Ms4a1[242]; Bank1, Ccr7, CD55, CD79a, CD79b, Col1a1, Fcmr, Ly6d, H2-Dmb2, H2-Ob, and Ms4a1[30,a] • Natural killer cells: CD3[243], CD16Natural killer cells: CD3[243], CD16[244], CD56[243], Klrb1c, CD56[243], Klrb1c[244], and Ncr1[245] , and Ncr1[245] • T cell: CD2[246],CD3e[247],CD4[248], and CD8[248],CD3e[247],CD4[248], and CD8[248] • Group2 Innate Lymphoid Cells (IL2): Areg, CD25, CD127, Gata3, and Rora[249] Group2 Innate Lymphoid Cells (IL2): Areg, CD25, CD127, Gata3, and Rora[249] • Non-cytotoxic innate lymphoid cells 2: CD3g, CD3d, CD3e, CD247, Il7r, Itk, Lat, Lef1, Skap1, and Tcf7[30,a]	Undefined
Mast cells	• FcεRI and Kit[250,251] • CD63 and CD203c[252]	Undefined
Granulocytes	• Ccr1[253] • Csf3r[254] • S100a9[255]	Undefined
Schwann cells	• Fabp7 and S100b[256] • Plp1[257]Plp1[257] • Cnp[258] • Aspa, Cd59a, Col1a1, Gfra3, Gpr37l1, Kcna1, Nrn1, and Stmn1[30,a]	Peripheral nervous system[256]

Glossary terms

Myofibroblasts: specialized fibroblasts that have developed some phenotypic and functional features of smooth muscle cells, including expression of smooth muscle actin and contraction capabilities upon stimulation.

Granulation tissue: highly vascularized connective tissue with granular projections, temporarily replacing lost tissue during repair.

Yolk sac: a membranous sac attached to the gut of an embryo and normally provides nutrition (yolk) to the developing embryo. In mammals, which are placental organisms, the yolk sac is part of the early circulatory system, linked to the primitive aorta. Primitive blood cells are formed as 'blood islands' in the yolk sac during early development (around 7 days of mouse development).

Plasma cells: Circulating mature B cells which produce large amounts of a specific antibody.

Memory T cells: Subset of T cells that previously encountered and responded to their cognate antigen, and provide rapid protection upon re-exposure to the same antigen due to enhanced function (memory of encountering an antigen) and lower activation threshold.

Regulatory T (T_{reg}) cells: Subset of CD4+ T cells which regulate/suppress other cells of the immune system, thus maintaining tolerance to self-antigens, and preventing autoimmune diseases.

Effector T cells: T cells (CD4+, CD8+, Treg cells) that actively respond to a stimulus, such as co-stimulation.

Autoreactive T cells: Subset of T-cells which have bypassed the negative selection in lymphatic organs and respond to self-antigen stimulation.

Tolerogenic phenotype: Phenotype of immune cells which are tolerant to a particular antigen.

Biological therapies: Treatments which make use of natural biological molecules, such as antibodies and growth factors.