Concise Review: Reduction of Adverse Cardiac Scarring Facilitates Pluripotent Stem Cell-Based Therapy for Myocardial Infarction

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ABSTRACT

Pluripotent stem cells (PSCs) are an attractive, reliable source for generating functional cardiomyocytes for regeneration of infarcted heart. However, inefficient cell engraftment into host tissue remains a notable challenge to therapeutic success due to mechanical damage or relatively inhospitable microenvironment. Evidence has shown that excessively formed scar tissues around cell delivery sites present as mechanical and biological barriers that inhibit migration and engraftment of implanted cells. In this review, we focus on the functional responses of stem cells and cardiomyocytes during the process of cardiac fibrosis and scar formation. Survival, migration, contraction, and coupling function of implanted cells may be affected by matrix remodeling, inflammatory factors, altered tissue stiffness, and presence of electroactive myofibroblasts in the fibrotic microenvironment. Although paracrine factors from implanted cells can improve cardiac fibrosis, the transient effect is insufficient for complete repair of an infarcted heart. Furthermore, investigation of interactions between implanted cells and fibroblasts including myofibroblasts helps the identification of new targets to optimize the host substrate environment for facilitating cell engraftment and functional integration. Several antifibrotic approaches, including the use of pharmacological agents, gene therapies, microRNAs, and modified biomaterials, can prevent progression of heart failure and have been developed as adjunct therapies for stem cell-based regeneration. Investigation and optimization of new biomaterials is also required to enhance cell engraftment of engineered cardiac tissue and move PSCs from a laboratory setting into translational medicine. STEM CELLS 2019;37:844–854

SIGNIFICANCE STATEMENT

This review focuses on interactions between implanted stem cells and fibroblasts after myocardial infarction (MI). Understanding of the process of cardiac scarring in the infarcted heart is important for design and timing selection of cell implantation in clinics. Potential effects of fibroblasts and collagen matrix remodeling on stem cells are discussed. Finally, this review proposes a combination of antifibrotic strategies and stem cell-based therapies for MI treatment. This research helps with identification of new targets that can optimize the host substrate environment for facilitating cell engraftment and functional integration.

INTRODUCTION

Myocardial infarction (MI) is an anemic infarct associated with cell death of myocardium and frequently causes heart failure or cardiac arrest [1]. The regenerative capacity of human cardiomyocytes is very limited and current pharmacotherapies do not offer an effective strategy for replenishing the lost cells during MI. As a result, necrotic tissue is replaced by scar formations composed of cardiac fibroblasts and collagens [2]. Although scar tissue can preserve structural integrity of the infarcted heart, it is still a desirable means of cardiac repair to attenuate collagen turnover by targeting the activated fibroblasts, because excessive collagen deposition in scar tissue has multiple adverse consequences such as cardiac atrophy and arrhythmogenicity [3, 4].

Stem cells with cardiogenic potential hold promise as a scalable cell source for cardiac regenerative therapy. Recent advances in bioengineering move us closer to a goal of generating functional heart tissue. Currently, pluripotent stem cells (PSCs) including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (iPSCs) are the main cell sources that can definitively generate cardiovascular cells (seed cells) in high quantities for cardiac tissue engineering [5]. Technology of engineered heart tissue (EHT) has made great strides in use of
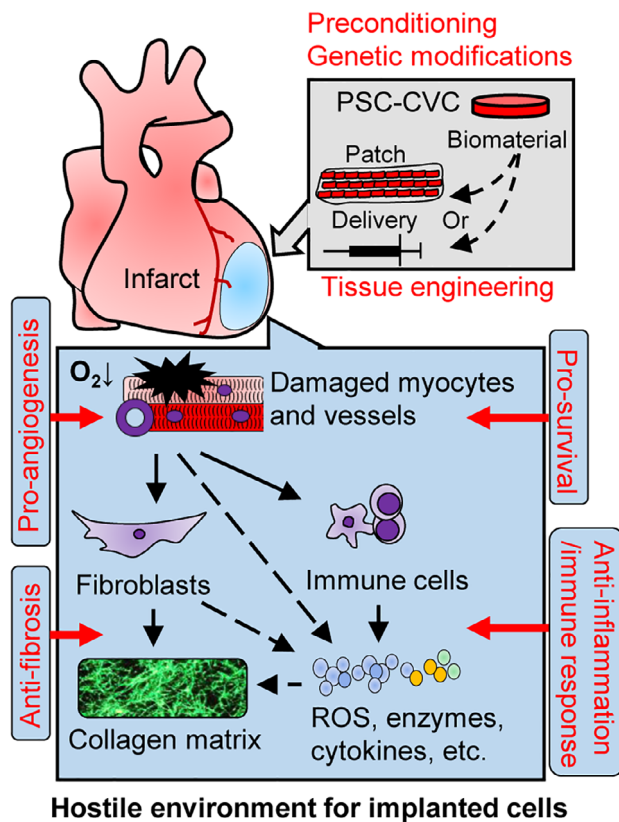
Figure 1. Strategies to improve survival and engraftment of implanted cells. Poor cell engraftment remains a major challenge for stem cell-based therapies such as use of pluripotent stem cells derived cardiovascular cells. Before cell implantation or delivery, there are various strategies available to evoke an adaptation and increased resistance to the hypoxic stimulus, including physical or chemical preconditioning, genetic manipulation, and use of biomaterial in tissue engineering to prevent cell damage. After implantation in an extremely low oxygen and nutrient ischemic environment, the graft cell survival will be challenged by pro-apoptotic reactive oxygen species, proteolytic enzymes, and pro-inflammatory or cytotoxic cytokines released from the damaged host heart tissue. And graft cell migration will be further limited by the excessive deposition of collagen matrix from fibrotic response. Accordingly, there are several strategies targeting the host environment in order to enhance cell engraftment and functional integration. Abbreviations: ROS, reactive oxygen species; PSC-CVC, pluripotent stem cells derived cardiovascular cells.

human iPSCs for modeling congenital heart diseases and drug test in vitro [6]. However, insufficient engraftment and integration with host tissue after transplantation remains a critical hurdle for clinical translation of using EHTs in regenerative therapy. Other issues including deficient vascularization, hostile ischemic environment, fibrotic scarring, and immune responses can influence the survival and cell fate of transplanted EHTs [7, 8]. Therefore, it is important to converge various anti-inflammatory, pro-survival, or pro-angiogenic strategies with tissue engineering technologies to overcome these challenges of developing PSC-based therapy (Fig. 1).

Poor cell engraftment remains a major challenge hindering application of stem cell-based therapies for MI. Recent studies have focused on manipulation of stem cells and optimization of delivery approaches to enhance cell survival and engraftment and address the safety issues in infarcted heart, as summarized

in other comprehensive reviews [9–11]. In this present review, we focus on the effect of host fibrotic environment on stem cell engraftment and highlight the importance of antifibrotic approaches for enhancing stem cell efficacy. Given that PSC are an attractive, reliable source for generating functional cardiomyocytes for heart regeneration, we also discuss new findings on the potential interaction between host cardiac fibroblasts, extracellular matrix (ECM), and implanted cardiomyocytes. Our previous studies have demonstrated that scar tissue is a physical barrier to the engraftment of implanted cardiac cell patches, whereas decreased collagen deposition in infarcted heart can increase cell engraftment and further enhance functional recovery [12, 13]. These findings support a potential feedback loop between cardiac fibrosis and cell engraftment (Fig. 2A) that is discussed in more detail below.

IMPACT OF CARDIAC FIBROSIS AND ECM REMODELING ON TRANSPLANTED CELLS

Fibroblasts are one of the most abundant resident noncardiac cells in the heart, and secrete ECM components such as collagens and fibronectin. Their primary function is to maintain ECM homeostasis through production and remodeling of collagens and other macromolecules including matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases [14, 15]. ECM also plays an important role in distributing mechanical stress in the heart, providing a scaffold for cardiovascular cells, and electrically insulating the ventricles from the atria [14, 16]. In normal heart, fibroblasts are nonexcitable cells and their functions are quiescent. Following MI, cardiac fibrosis or scarring is a result of excess ECM production by myofibroblasts that are derived from fibroblasts activated by inflammatory growth factors and cytokines, such as tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β [17]. The adverse ECM remodeling in scar is a detrimental mechanism contributing to the progression of heart failure [18]. Components and organization of the newly synthesized ECM in infarcted heart are different from those of native ECM [19]. During different phases of healing after MI, dynamic changes in the composition of ECM play a critical role in regulation of cellular biological responses that mediate engraftment of transplanted cells. Therefore, a better understanding of the process of cardiac scarring in infarcted heart is important for design and timing selection of cell transplantation in patients. In terms of infarct healing processes (including inflammatory, proliferative, and maturation phases) [20], the potential effects of fibroblasts and ECM network on engraftment of stem cells (including PSC-derived cardiovascular cells) are discussed in this section (Fig. 2B).

Early Inflammation Impairs Survival of Transplanted Cells

Large amounts of MMPs (including collagenases and gelatinases) are synthesized de novo by cardiovascular cells and the infiltrating leukocytes in acutely infarcted hearts [21]. Occurrence of ischemia causes rapid activation of latent MMPs and early degradation of ECM proteins, generating bioactive fragments (termed matrikines) that contribute to activation of immune cells and inflammatory pathways [22, 23]. The role of endogenous matrix fragments in regulation of angiogenic responses and graft–host integration following cell transplantation remains
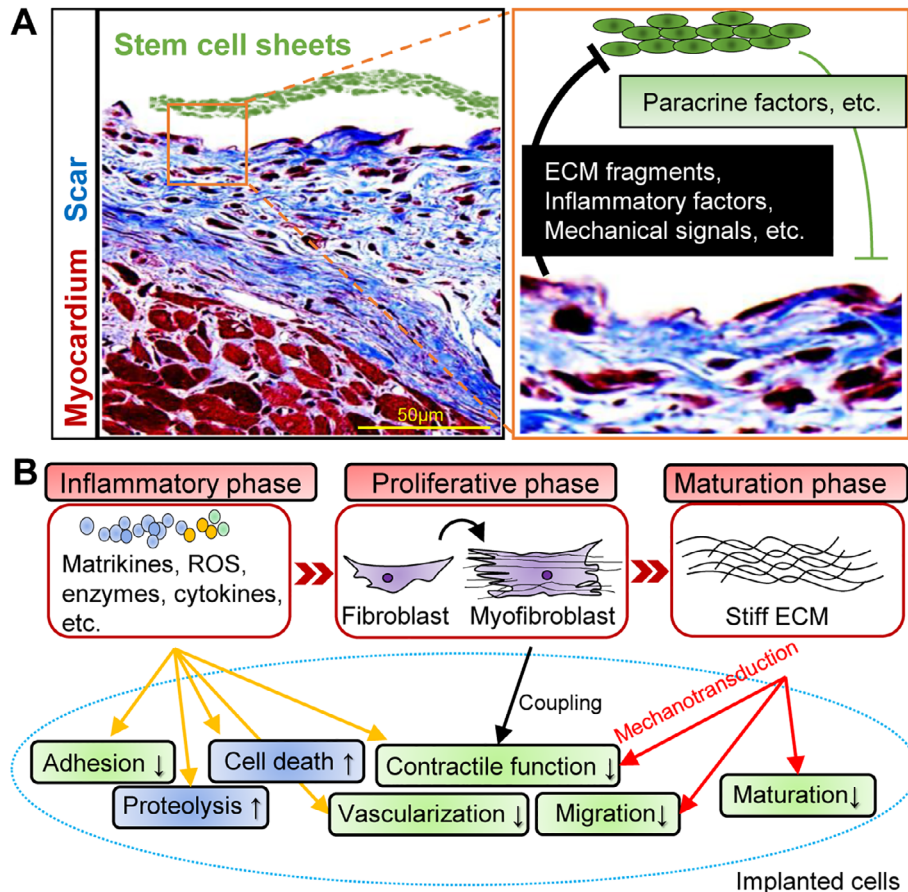
Figure 2. Potential interactions between fibrotic tissue and implanted cells. **(A):** Representative Masson's trichrome imaging of myocardial scar tissue at 1-week postinfarction. Following infarct healing, damaged myocardium is rapidly replaced by fibrotic tissue composed of fibroblasts and collagens. Implanted cells (either epicardial or intramyocardial delivery) invade in fibrotic tissue through production of paracrine factors to degrade fibrillar collagens. Conversely, multiple factors, such as fragments of extracellular matrix, pro-inflammatory cytokines, and mechanical signals, derived from fibrotic tissue can obstruct the migration and engraftment of implanted cells. Scale bar = 50 μm. **(B):** During post-MI inflammatory phase, the survival and function of implanted cells is repressed by multiple pro-inflammatory factors. During the overactive reparative or proliferative phase, the electrophysiological activity of implanted cardiomyocytes would be interrupted by the myofibroblasts through gap junction or mechanical coupling. During the maturation phase, the increased tissue stiffness would inhibit the contractile function of implanted cardiomyocytes through mechanotransduction signaling. Vascularization, cell migration, and structural maturation of implanted cardiac cells would be inhibited in the fibrotic environment. Abbreviations: ROS, reactive oxygen species; ECM, extracellular matrix.

poorly understood. Inflammatory cytokines (e.g., TNF-α, IL-1β, and IL-6) and reactive oxygen species that are elevated in MI can change the electrophysiological activities of cardiomyocytes and increase the risk of ventricular arrhythmia [24]. Moreover, MMPs can regulate inflammatory responses and cellular migration through proteolytic processing of cytokines or growth factors or by degrading glycosaminoglycan binding sites [21, 25], thereby interrupting the vascularization of EHT that is mediated by chemokine immobilization of endothelial cells. Tissue adhesion of transplanted cells can be reduced by the process of proteolytic cleavage and ECM clearance [26]. MMPs also have been reported to degrade intracellular cardiomyocyte proteins such as myosin, α-actinin, and titin [27], and therefore possibly damaging the structural integrity of EHT. The role of matrix-independent actions of MMPs in adhesion, survival, and function of stem cells remains to be addressed.

Infiltration of inflammatory leukocytes, release of proinflammatory cytokines, and production of proteases in the harsh milieu during inflammatory phase present a formidable roadblock to cell-based cardiac repair. Additionally, formation of a provisional matrix network derived from extravasated plasma proteins can provide a highly plastic conduit for infiltrating immune cells and serve as a reservoir for inflammatory cytokines and growth factors [19]. However, few studies systematically determine the influence of these factors on survival and engraftment of stem cells. In murine MI models, the intramyocardial infiltration of CD45+ leukocytes including neutrophils and macrophages reached a maximum at 4–7 days following ligation surgery, and most of implanted cells did not survive in the acute inflammatory environment [28]. Leukocyte-released oxygen-derived free radicals, proteases, and inflammatory cytokines that can directly damage cardiovascular cells via cell membrane damage, proteolysis, or activation of cell death caspase signaling pathways [29, 30]. Post-MI inflammation is most severe in acutely injured heart, but could also have a considerable effect on cell graft survival, in addition to ischemic stress. Although these cell death processes possibly recapitulate response of implanted cells to the inflammatory factors, the influence of inflammation

on graft survival has not been determined in infarcted heart. Caspase-dependent or caspase-independent cell death events including cell swelling, increased membrane permeability, and cell rupture [31, 32] may occur after inflammatory cytokines (such as TNF-α and IL-1β) binding to their receptors on transplanted myocytes. Investigation of inflammasome-triggered cell death is also important for development of cell-protective approaches for enhancing graft survival.

Activation of Myofibroblasts Interrupts Electrical Coupling of Transplanted Cells

Infarct healing transitions from the inflammatory to the proliferative phase after removal of dead cells and matrix debris by phagocytes and release of anti-inflammatory mediators [33]. At the proliferative phase, the remodeled ECM networks do not serve a primary structural role but provide essential signals such as TGF-β, hyaluronan, and versican for conversion of cardiac fibroblasts into myofibroblasts [34, 35]. Myofibroblasts are the dominant cells in the proliferative phase and have characteristics of fibroblasts and smooth muscle cells, such as producing large amounts of collagens, expressing α-smooth muscle actin (SMA), and the ability to contract [36]. Myofibroblasts typically generate a sustained contraction resembling that of smooth muscle cells in response to mechanical stretch, autocrine, and paracrine factors within the myocardium (such as angiotensin II and TGF-β) and hormones derived from the circulation (such as aldosterone) [37]. The nature of contraction generated by myofibroblasts is fundamentally distinct from that generated by cardiomyocytes that contract and relax cyclically following a stimulus from the cardiac conduction system through electric coupling [37]. Therefore, electrophysiological functions of cardiomyocytes will be impaired by the myofibroblast-derived contractile forces transmitted to the cardiomyocyte membrane and activation of mechanosensitive channels [38]. Moreover, the α-SMA microfilaments expressing in myofibroblasts contribute to abnormal automaticity and impulse conduction, which adversely affects the electrical properties of cardiomyocytes and then enhances the arrhythmogenic propensity through gap junctions [3, 39]. Mechanical coupling between myofibroblasts and cardiomyocytes plays a prominent role in damaged heart [38]. Thus, myofibroblasts are likely to interrupt electrical coupling of transplanted PSC-derived cardiomyocytes in infarcted heart.

Several recent studies have focused on the coupling between myofibroblasts and transplanted EHT. Methods of in vitro two-dimensional (2D) or three-dimensional (3D) coculture system have been established to mimic arrhythmogenic myocyte-fibroblast communications [40], which serves as a platform to investigate the electrical coupling of EHT. Proliferation and gap junction of myofibroblasts plays an important role in the conduction abnormalities of cocultured neonatal rat cardiomyocytes [41, 42], suggesting myofibroblasts as a potential target to reduce cardiac arrhythmias. Furthermore, the impaired electrical propagation in monolayers of cocultured neonatal rat cardiomyocytes and myofibroblasts was reversed by engraftment of hESC-cardiomyocytes via gap junction coupling (connexin-43) to the rat cardiomyocytes [43]. The heterocellular coupling of hESC-cardiomyocytes to the rat cardiomyocytes was not interrupted by the cocultured myofibroblasts that were predominately found underneath the rat cardiomyocytes in the monolayers without directly contacting hESC-cardiomyocytes [38, 43]. Interestingly,

Torsade de Pointes-like arrhythmias can be observed in 3D iPSC-EHT with coculture of cardiomyocytes and mesenchymal nonmyocytes but not in pure cardiomyocytes under the treatment of K+ channel blockers [44]. These studies reveal important insights into the interactions between myofibroblasts and host cardiomyocytes, and are a likely explanation for the potential electrical activity of newly implanted cardiomyocytes. Although an in vitro coculture system can provide visualized evidence for electrical coupling of cardiomyocytes, it needs further optimization to delineate the influence of fibrotic tissue on electromechanical features of PSC-cardiomyocytes. For instance, isotropic cell distribution patterns and paracrine effects of mesenchymal cells can affect the conduction velocity and excitability of cardiomyocytes [45]. Additionally, use of human fibroblasts, change of cell dosage or ratio, cell distribution/engraftment patterns, modification of ECM components, and long-term culture should be considered for future study.

It is still difficult to determine the heterogeneous cell coupling between PSC-cardiomyocytes and host cells in situ due to the uncertainty of the cellular component and density at infarct or remodeled peri-infarct zones where cells were transplanted or injected. Susceptibility to arrhythmia in the infarcted heart is associated with the density of myofibroblasts that serve as a current sink or source [46, 47]. Interestingly, there were fewer arrhythmias in small infarct models than in larger infarct models before cell delivery, whereas intramyocardial injection of hESC-cardiomyocytes dramatically triggered arrhythmias in the former but did not further increase the severity of arrhythmias in the latter [48, 49]. This discrepancy suggests that the electrophysiological consequences of infarcted myocardium may be dependent on the ratio of myofibroblasts to implanted myocytes. Although an implantable defibrillator can prevent the life-threatening arrhythmias event in patients [50], studies evaluating the optimal cell dosage in relationship to infarct size are warranted to further address safety issues. Therefore, myofibroblasts are involved in the electromechanical coupling between implanted cells and host cells during the proliferative phase of MI and the underlying cellular mechanisms remain to be investigated in various animal models.

Stiff Matrices Impede Cell Migration and Contraction

Following the proliferative phase, the maturation phase is marked by a reduction of inflammatory and reparative cells (such as myofibroblasts) and maturation of scar tissue and remodeling. Fibrous scar tissue is predominantly composed of type I collagen that has a tensile strength and prevents ventricular wall rupture [3, 51]. Excessive collagen-I synthesis increases myocardial stiffness and generates higher cell-matrix tension and a more definitive pro-contractile tissue environment [52]. Collagen turnover in the maturation phase is maintained by the remaining myofibroblasts, and pro-migratory, soft collagens (such as collagen-III) are replaced with collagen-I [53]. Moreover, collagen-I can be further modified by lysyl oxidases-catalyzed crosslinking thereby increasing tissue stiffness [54]. Excessive collagen deposition and increased myocardial stiffness promotes diastolic and systolic dysfunction in MI [55]. Tissue rigidity of adult heart is ~10 kPa but will be increased above 100 kPa after cardiac fibrosis [56]. These findings of excessive collagen deposition recapitulate the microenvironment in which the engineered cardiovascular cells are injected or delivered.

The subtype, quantity, and density of collagens determine the mechanical properties of ECM in fibrotic tissue [52], thereby affecting the functions of contacted cells. For instance, electrical communication, structural maturation, and contractile function of hESC-cardiomyocytes were limited in high-density collagen-I matrix, but were not impacted by low collagen-I concentrations [57]. Similar inhibitory responses were found in isolated embryonic cardiomyocytes on hard, stiff matrices [58]. Furthermore, intercellular network formation, sarcomeric structure maturation, contraction velocity, and contractile function can be enhanced by optimization of matrix stiffness [59]. Interestingly, the expression of α actinin and myosin heavy chain in typical striations and the upregulation of sarcomeric actin were found in cardiomyocytes cultured on softer substrates, whereas preferential expressions of immature cardiac cell genes and inflammatory genes were detected in cardiac cells grown on stiffer materials [60]. Other in vitro models showed that the cell proliferation, migration, and paracrine effects of cardiosphere-derived cells were inhibited by the ECM derived from diseased hearts [61, 62]. These findings on the influence of collagen density on functions of cardiomyocytes have been applied in cardiac tissue engineering, but few studies focus on the relationship between transplanted cells and fibrotic tissues. The local cardiac ECM network would serve as a scaffold for attachment of transplanted cells, yet it is unknown whether increased tissue stiffness in vivo is detrimental for cell penetration, migration, and functional interaction. Therefore, characterization of ECM components (including collagens and other matricellular proteins) in a future study is important to demonstrate the influence of scar tissue on stem cell engraftment and therapeutic actions.

Scar Tissue Alters Cardiomyocyte Contractility via Mechanotransduction

Mechanisms underlying the change of stem cell fate (e.g., mesenchymal stem cells [MSCs] and iPSCs) in response to ECM elasticity or stiffness have been extensively studied [63, 64]. Cells can sense and respond to ECM stiffness via mechanisms of mechanotransduction [65]. Cell behaviors (including morphology changes, survival, proliferation, and migration) can be mediated by ECM stiffness through molecular mechanosensors and transducers (e.g., integrins and actin cytoskeleton) [65, 66]. Investigation of mechanotransduction between ECM and cardiomyocytes will elucidate the adhesion mechanism of engineered PSC-derived cardiovascular cells in infarcted heart. Integrins, that are heterodimeric surface receptors, provide for cellular adhesion and also act as mechanotransducers connecting the cytoskeleton (or muscle sarcomere) with the ECM [67]. Integrins have been shown to bind adaptor proteins such as talin and vinculin to regulate both mechanical and electrical coupling of cardiomyocytes through linking the myofibrils to the ECM [68, 69]. A computational pathway model has been established to reveal key signaling regulators and downstream genes of mechanosensors including integrins, angiotensin type 1 receptor, and calcium channels in the cardiac mechano-signaling network [70]. Therefore, the response of cardiomyocytes to stiffness is dependent on expression of myofibrillar proteins and the assembly and maturation of myofibrils [69, 71]. Interestingly, nonmuscle myosin induced force generation or contractility also plays a pivotal role in cardiomyocytes sensing matrix rigidity [72]. In stiffer heart tissue, protein kinase C (PKC) was activated as an intrinsic regulator of nonmuscle myosin activity and increased nonmyofibrillar contractility, thereby interrupting integrin-talin associated myofibrillar contraction [72]. This mechanism explains the contribution of aberrant mechanosensing to long-term remodeling in heart failure and suggests the nonmyofibrillar force signaling as a potential pharmacological target for future study. Since the nonmuscle myosin plays a central role in the control of cell adhesion and migration [73], its role in cell engraftment of implanted stem cells particularly for PSC-cardiomyocytes needs further investigation in stiff, fibrotic heart.

Supramolecular Fibrillar Structures Harm Vascularization

In vivo graft survival is dependent upon sufficient nutrient and oxygen supply via vascularization. Vascular cells can degrade basement membrane through secretion of MMPs and then invade and form vessels in ECM [74]. Increased myocardial stiffness can activate angiogenic potential of endothelial cells in diseased heart [75]. However, simply seeding the vascular cells in EHT may not be sufficient to ensure a good vascular organization and tissue perfusion after transplantation in the long-term [76]. Moreover, cardiac angiogenic capacity is lost in the ischemic area replaced by scar tissue [77, 78]. Vascular integration processes including cell proliferation, migration, and sprouting would be remodeled or disorganized in response to the dynamic changes of ECM proteins during cardiac fibrosis. Copolymers organized by excessive accumulation of crosslinked collagen-I may present a physical barrier that impairs migration of transplanted cells or limits invasion of microvessels of host origin. Fibrotic tissue also expresses several antiangiogenic or pro-apoptotic factors such as angiotensin-II and TGF-β1 [78, 79]. Although the initial length and diameter of vascular structures can be designed and controlled in 3D engineered tissue [76], it is likely that the well-organized network will soon revert to a random organization after implantation when there are no additional cues in scarred myocardium to guide the vascular remodeling process. Since poorly organized and immature vessels cannot supply sufficient nutrients, the quality of vascular network is also more important than the quantity [80]. An exact prediction of the resulting vascular network and geometry is still challenging due to the complexity of environmental factors. Therefore, proper manipulation of the environment factors will facilitate the vascular inosculation between EHT and host myocardium. In addition, rapid in vivo vascularization of EHT and efficient stimulation of de novo angiogenesis are important approaches to ensure survival of implanted cells. To this end, techniques are being developed for connecting prevascularized grafts to host vasculature within a short time, such as microsurgical anastomosis, biodegradable scaffolds, 3D printing graft architecture, and supplement of angiogenic growth factors [81–83].

ANTIFIBROTIC EFFECT OF STEM CELLS

Each phase of cardiac scarring affects the therapeutic potential of stem cell-based therapy as discussed above. Conversely, these exogenous cells can reduce cardiac fibrosis in a beneficial manner, and implantation of PSC-derived EHT has been reported to replenish infarct scar [84]. A prevailing perception is that implanted cells exert paracrine effects such as expressing cytokines and MMPs to promote degradation of collagens in scar tissue, which has
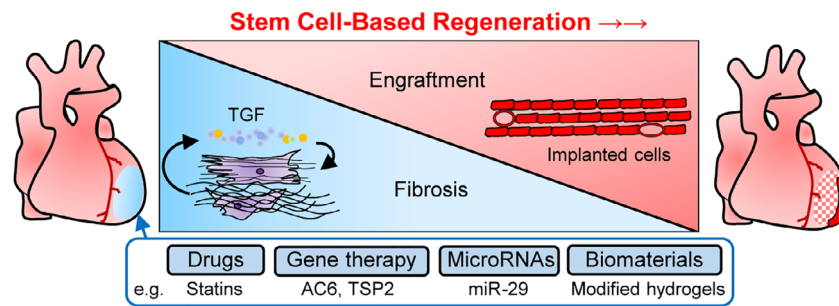
Figure 3. Overview of antifibrotic approaches to enhance stem cell-based regeneration. An antifibrotic strategy targeting the fibrogenic signaling (such as TGF) and collagen deposition can be applied to enhance the engraftment of implanted stem cells or pluripotent stem cell-derived cardiac cells. Multiple approaches such as using pharmacological agents, gene therapy, microRNA mimics, and modified biomaterials are exemplified. Abbreviations: TGF, transforming growth factor; AC6, adenylyl cyclase-6; TSP2, thrombospondin-2.

been heavily discussed in other reviews [85, 86]. However, the cell–cell interaction between implanted cells and host cells including myocytes and fibroblasts is not fully understood. Formation of connexin junctions or tunneling nanotubes between cardiomyocytes and fibroblasts also contributes to the electrical activity of nonmyocytes at the scar border [87, 88]. Nanotubular crosstalk with cardiomyocytes has been found to enhance the paracrine effects of MSCs as evidenced by higher angiogenesis and stem cell homing at the infarction site [89]. It is likely that implanted PSC-derived cardiac cells can directly regulate the host cells or fibroblasts in infarcted heart through various intercellular connections, but this possibility remains to be demonstrated. Interactions between donor cells and recipient tissue are complicated and need more investigation. It is presumed that implanted cells can reduce cardiac fibrosis and that reduction of scar formation will allow for enhanced cell migration and engraftment, ultimately establishing a positive feedback loop to remuscularize infarcted heart. Such insights of mechanism of actions will provide a basis for future design of new MI treatments using more efficient cell types and targeting cell–cell interactions aiming for a reversal of malignant fibrosis and preventing heart failure.

## CONVERGENCES OF ANTIFIBROTIC STRATEGIES AND CELL-BASED THERAPY

Despite encouraging results from large animal studies of using PSC-cardiomyocytes [48, 49], translation toward clinical application is hampered by several issues such as low graft survival and poor functional integration and engraftment. Long-term persistence of myofibroblasts in ischemic heart interrupts infarct healing and contributes to excessive deposition of ECM that could limit the efficacy of the contractile units in EHT. In addition to research efforts in protecting donor cells, manipulation of fibrotic environment to which cells will be delivered should be considered in order to enhance the vascularization and functional integration (Fig. 3).

Pharmacological Approaches Mediating Cardiac Fibrosis

Pharmacological interventions inhibiting or reversing fibrosis and its adverse consequences are widely used for treating heart disease due to a favorable safety profile, such as angiotensin-converting enzyme inhibitors and statins [90]. Although these
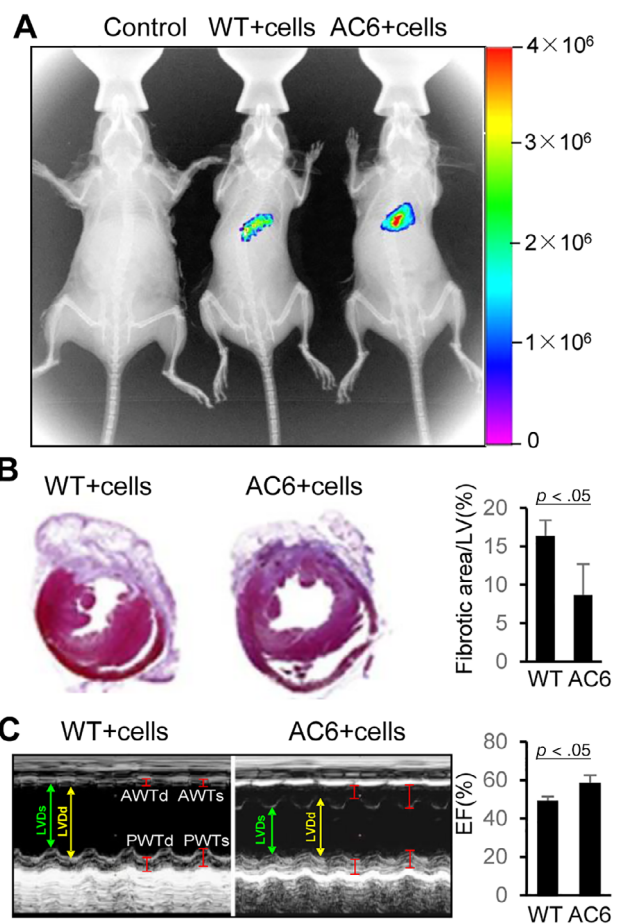
Figure 4. Overexpression of AC6 facilitates cell engraftment of induced pluripotent stem cell (iPSC)-derived patches. **(A):** In vivo engraftment of iPSC-derived cardiac cell patches in WT or AC6 transgenic mice was tracked by bioluminescent imaging assay at 4 weeks after surgery. A stronger cell engraftment signal was observed in AC6 mice as compared with WT mice. WT mice were implanted with cell patch without reporter gene as a background control. **(B):** Heart sections at 4 weeks after cell patch transplantation stained with Masson's trichrome and quantification of the percentage of LV fibrotic area. **(C):** M-mode echocardiograms in WT or AC6 mice implanted with cell patch. Quantitative data of EF up to 4 weeks after surgery. Abbreviations: AC6, adenylyl cyclase-6; WT, wild-type; LV, left ventricular; EF, ejection fraction (reprinted with permission from Dai et al. J Am Coll Cardiol 2011;58:2118).
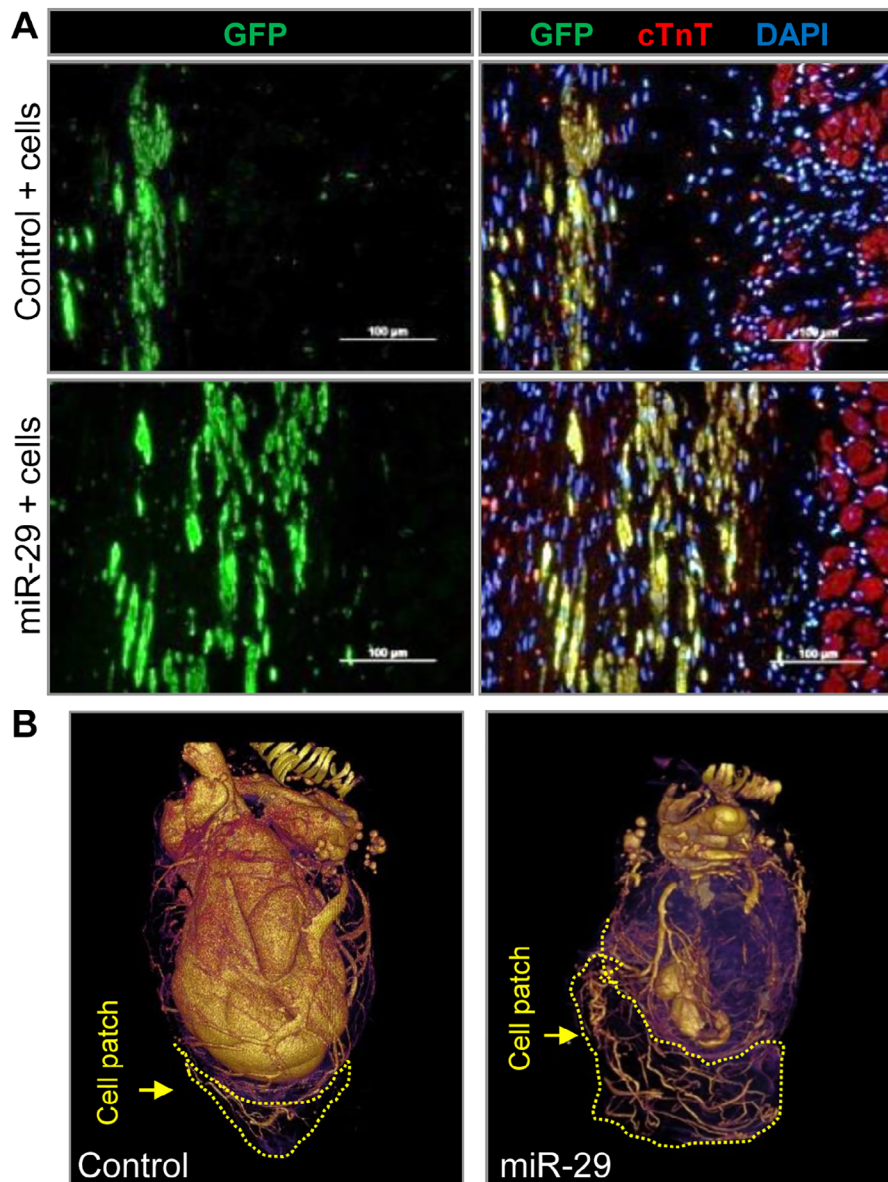
Figure 5.   Use of miR-29 mimic facilitates cell migration and vascularization of induced pluripotent stem cell (iPSC)-derived patches. **(A):** Migration of iPSC-derived cells labeled by GFP (green) in infarct zone was enhanced after administration of miR-29 mimic in myocardial infarction (MI) mice. Viable cardiomyocytes derived from iPSC (yellow) or host origin (red) were identified by staining of cardiac troponin T (cTnT). Scale bars = 100 μm. **(B):** Perfusion of contrast agents in micro-CT imaging indicates the cardiac collateral vessel networks of MI mice that were treated with miR-29 mimic or negative control during cell implantation. The dotted line shows the location of cell patch (reprinted with permission from Huang et al. PLoS One 2013;8:e70023).

interventions contribute to a partial recovery of contractile dysfunction, the progression toward heart failure will not be reversed unless the lost cardiomyocytes can be fully regenerated. Simultaneous application of stem cell-based regenerative medicine and pharmacotherapies emerges as a promising strategy to replenish the lost myocardium and restore heart function. Use of statins in combination with cellular therapy (such as MSCs) appears promising in preclinical studies, but has not been well studied in human [91]. Administration of statins in MI models was shown to reduce apoptosis and enhance migration and survival of implanted stem cells through activation of multiple pathways such as SDF-1/CXCR4, AKT, and ERK [92–94]. Statins can be used as an adjunct

treatment to enhance the therapeutic efficacy of adult stem cells, while the mechanism of actions remains ambiguous. Ischemic myocardium during the inflammatory phase may be improved by the antioxidant and anti-inflammatory properties of statins [95]. Statin treatment also can protect endothelial barrier function, thereby reducing infiltration of inflammatory leukocytes in infarcted heart [96]. In addition, treatment of simvastatin was shown to inhibit differentiation of cardiac fibroblasts into myofibroblasts activated by TGF-β1 [97]. These findings suggest that early use of statins during the acute inflammatory phase can provide a favorable microenvironment for graft survival in infarcted heart. Intriguingly, cardiac gene expression and cardiomyogenesis

of ESCs were enhanced by simvastatin treatment [98]. Regarding the common use of statins in patients with heart disease and their pleiotropic effects on stem cell biology [99, 100], further investigation of statins affecting survival and engraftment of PSC-derived cardiovascular cells has great clinical implications.

Manipulation of Fibrosis-Associated Target Genes

There is increasing evidence that engraftment and survival of implanted cells in infarcted heart will be enhanced by targeting the genes controlling ECM protein expression and matrix remodeling. Studies have demonstrated the feasibility and safety of strategies that directly target collagen turnover and organization. The use of antifibrotic hormone, relaxin, has less risk of post-MI ventricular wall rupture or left ventricular (LV) remodeling and offers great potential benefits for stem cell based therapies for MI treatment [101]. Overexpression of adenylyl cyclase-6 (AC6) has been shown to inhibit fibroblast activation and collagen synthesis through targeting TGF-β signaling pathways [102]. Our previous study showed that cell engraftment of PSC-cardiac cells was significantly increased after reduction of collagen deposition in AC6 transgenic mice [12]. Moreover, improvement of heart function contributing to cell therapy was more prominent in AC6 transgenic mice than in controls (Fig. 4). Thrombospondin-2 is a pro-fibrotic, antiangiogenic matricellular protein, and its gene knockdown has been shown to improve cardiac graft integration, vascularization, and survival [103]. Gene therapy targeting fibrogenic pathways has the potential to reduce the scarring obstacle around cell injections and then improve graft integration in cell-based cardiac repair. This promising approach combining gene therapy and stem cells needs further investigations in new delivery vehicles, tissue targeting specificity, and immune response.

MicroRNAs also play an important role in the regulation of cardiac fibrosis by targeting the pathogenic pathways [104]. For instance, miR-29 family has been implicated in cardiac fibrosis through targeting multiple mRNAs that encode ECM proteins such as collagens, fibrillins, and elastin [105]. Our previous study aimed to develop overexpression of miR-29 as an antifibrotic approach to facilitate stem cell survival and engraftment [13]. We found that injection of miR-29 mimics significantly enhanced migration and vascularization of implanted iPSC-derived cell patches in infarcted heart (Fig. 5). Recently, a phase 1 clinical trial has been completed to assess whether MRG-201 (a miR-29 mimic) can be safely used as an antifibrotic therapy in certain diseases (NCT02603224). Therefore, the technically feasible microRNA supplementation or antisense oligonucleotides hold promise as a viable complementary agent for stem cell-based therapies in heart diseases with formation of fibrous scar tissue.

Antifibrotic Biomaterials for Building EHT

Biomaterials have been used to facilitate all steps of PSC production including expansion and differentiation protocols [106]. Implantation of EHT, which combines PSC-derived cardiovascular cells and biomaterials, is a promising therapeutic method for regeneration of infarcted heart. To obtain mature EHT in drug screening or cell therapy, natural biomaterials or synthetic nanomaterials (such as alginate, fibrin, collagen, and polylactic-coglycolic acid) have been used to provide mechanical support and generate 2D or 3D cardiomyocyte sheets with noncontractile cells [107–110]. However, there are many obstacles faced by investigators in translating the applications of biomaterials to the clinic [111]. It is still challenging to identify ideal biomaterials that closely simulate natural ECM for cell survival, allow cell migration into infarct zone, strengthen donor-host cell coupling, and have no immune responses after degradation. In this section, we focus on the antifibrotic biomaterials that have the potential to enhance cell migration and engraftment in preclinical studies.

Although several biomaterials have been identified to reduce tissue stiffness or inhibit cardiac fibrosis, it remains to address whether they can be synergistically used in PSC-based therapies. Microstructures of hyaluronic acid or hydrogel (fabricated by photolithography) were shown to anchor fibroblasts and mitigate cardiac fibrosis [112, 113]. MMPs (e.g., MMP2, MMP9, and MMP13) are required to correctly degrade the implanted scaffolds and endogenous natural ECM, allowing for stem cell migration and angiogenesis [114, 115]. Furthermore, antifibrotic factors such as fibroblast growth factor-2 and hepatocyte growth factor can be coated with hydrogels or scaffolds that have been tested in infarcted heart [116, 117]. However, these modified materials have not been explored with cell transplantation. Studies of adult stem cells such as MSCs have demonstrated that proper biomaterials decrease scar formation, deliver growth factors, and offer biochemical cues important for cell migration and engraftment [8, 118]. Localized delivery of antifibrotic biomaterials has the potential to address the issue of low dosage and efficacy associated with systemic delivery of drugs [118]. Therefore, use of antifibrotic biomaterials potentially provides an effective delivery approach for stem cells and engineered tissues in the fibrotic microenvironment. Immunogenic, biocompatible, and mechanical properties of antifibrotic biomaterials should be further investigated and optimized in order to improve functional maturation of PSC-derived cardiovascular tissue.

CONCLUSION

Implantation of PSC-derived functional cardiovascular cells has great potential as a therapeutic for patients after MI. Postinfarct cardiac fibrosis can maintain structural integrity of the heart and reduce the risk of ventricular dilation and rupture, whereas matrix remodeling and formation of the avascular scar tissue impairs engraftment and survival of implanted cells in the damaged myocardium. The implanted cells would be inhibited by bioactive fragments of ECM proteins, immune cells, and pro-inflammatory cytokines during early fibrotic phase. Electrical coupling of transplanted cells can be disturbed by myofibroblasts with enhanced arrhythmogenicity. Stiff fibrous tissue can impede cell migration and contraction through altered mechanotransduction patterns. Moreover, vascularization of cell graft is limited in the hostile fibrotic environment with enriched antiangiogenic or pro-apoptotic factors. Although paracrine factors produced by implanted cells can improve cardiac fibrosis and cell survival, the transient effect is insufficient to repair infarcted heart as shown in clinical trials and large animal studies [86].

Development of EHT technology using PSCs and bioengineering will provide mature myocytes that replenish the lost myocardium, repair scar tissue, and are able to bear the local mechanical and hemodynamic load placed on them. In addition to efforts on manipulation of EHT ex vivo, we attempt to optimize the host substrate environment by targeting fibroblast activation pathways or modifying the ECM, in order to facilitate

cell engraftment and functional integration of newly regenerated cardiomyocytes. Several antifibrotic approaches including pharmacological agents, gene therapies, microRNAs, and modified biomaterials can be combined in stem cell-based therapies. And these intervention approaches require further investigations when combined with EHT technology to ensure protection against infarct expansion, ventricular rupture, and other potentially devastating post-MI complications [18, 20]. Targeting fibrosis not only prevents progression of disease toward heart failure, but also is a complementary therapeutic for cardiac regeneration.

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AUTHOR CONTRIBUTIONS

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this review article as no new data were created or analyzed in this study. All discussion is based on the cited references.

REFERENCES

1 Thygesen K, Alpert JS, Jaffe AS et al. Third universal definition of myocardial infarction. J Am Coll Cardiol 2012;60:1581–1598.

2 Jugdutt BI. Limiting fibrosis after myocardial infarction. N Engl J Med 2009;360: 1567–1569.

3 Weber KT, Sun Y, Bhattacharya SK et al. Myofibroblast-mediated mechanisms of pathological remodelling of the heart. Nat Rev Cardiol 2013;10:15–26.

4 Leask A. Getting to the heart of the matter: New insights into cardiac fibrosis. Circ Res 2015;116:1269–1276.

5 Yoshida Y, Yamanaka S. Induced pluripotent stem cells 10 years later: For cardiac applications. Circ Res 2017;120:1958–1968.

6 Merkle FT, Eggan K. Modeling human disease with pluripotent stem cells: From genome association to function. Cell Stem Cell 2013;12: 656–668.

7 Wu R, Hu X, Wang J. Concise review: Optimized strategies for stem cell-based therapy in myocardial repair: Clinical translatability and potential limitation. STEM CELLS 2018; 36:482–500.

8 Segers VF, Lee RT. Biomaterials to enhance stem cell function in the heart. Circ Res 2011;109: 910–922.

9 Sanganalmath SK, Bolli R. Cell therapy for heart failure: A comprehensive overview of experimental and clinical studies, current challenges, and future directions. Circ Res 2013;113:810–834.

10 Shafiq M, Jung Y, Kim SH. Insight on stem cell preconditioning and instructive biomaterials to enhance cell adhesion, retention, and engraftment for tissue repair. Biomaterials 2016;90:85–115.

11 Oikonomopoulos A, Kitani T, Wu JC. Pluripotent stem cell-derived cardiomyocytes as a platform for cell therapy applications: Progress and hurdles for clinical translation. Mol Ther 2018;26:1624–1634.

12 Dai B, Huang W, Xu M et al. Reduced collagen deposition in infarcted myocardium facilitates induced pluripotent stem cell engraftment and angiomyogenesis for improvement of left ventricular function. J Am Coll Cardiol 2011;58: 2118–2127.

13 Huang W, Dai B, Wen Z et al. Molecular strategy to reduce in vivo collagen barrier promotes entry of NCX1 positive inducible pluripotent stem cells (iPSC(NCX(1)(+))) into ischemic (or injured) myocardium. PLoS One 2013;8:e70023.

14 Camelliti P, Borg TK, Kohl P. Structural and functional characterisation of cardiac fibroblasts. Cardiovasc Res 2005;65:40–51.

15 Fan D, Takawale A, Lee J et al. Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease. Fibrogen Tissue Repair 2012;5:15.

16 Kakkar R, Lee RT. Intramyocardial fibroblast myocyte communication. Circ Res 2010; 106:47–57.

17 Hartupee J, Mann DL. Role of inflammatory cells in fibroblast activation. J Mol Cell Cardiol 2015;93:143–148.

18 Segura AM, Frazier OH, Buja LM. Fibrosis and heart failure. Heart Fail Rev 2014;19: 173–185.

19 Frangogiannis NG. The extracellular matrix in myocardial injury, repair, and remodeling. J Clin Invest 2017;127:1600–1612.

20 Ertl G, Frantz S. Healing after myocardial infarction. Cardiovasc Res 2005;66:22–32.

21 Alfonso-Jaume MA, Bergman MR, Mahimkar R et al. Cardiac ischemia-reperfusion injury induces matrix metalloproteinase-2 expression through the AP-1 components FosB and JunB. Am J Physiol Heart Circ Physiol 2006;291: H1838–H1846.

22 Wells JM, Gaggar A, Blalock JE. MMP generated matrikines. Matrix Biol 2015;44-46: 122–129.

23 Etoh T, Joffs C, Deschamps AM et al. Myocardial and interstitial matrix metalloproteinase activity after acute myocardial infarction in pigs. Am J Physiol Heart Circ Physiol 2001; 281:H987–H994.

24 Francis Stuart SD, De Jesus NM, Lindsey ML et al. The crossroads of inflammation, fibrosis, and arrhythmia following myocardial infarction. J Mol Cell Cardiol 2016;91:114–122.

25 Cox JH, Dean RA, Roberts CR et al. Matrix metalloproteinase processing of CXCL11/I-TAC results in loss of chemoattractant activity and altered glycosaminoglycan binding. J Biol Chem 2008;283:19389–19399.

26 Kirmse R, Otto H, Ludwig T. Interdependency of cell adhesion, force generation and extracellular proteolysis in matrix remodeling. J Cell Sci 2011;124:1857–1866.

27 Ali MA, Fan X, Schulz R. Cardiac sarcomeric proteins: Novel intracellular targets of matrix metalloproteinase-2 in heart disease. Trends Cardiovasc Med 2011;21:112–118.

28 Swijnenburg RJ, Govaert JA, van der Bogt KE et al. Timing of bone marrow cell delivery has minimal effects on cell viability and cardiac recovery after myocardial infarction. Circ Cardiovasc Imaging 2010;3:77–85.

29 Swirski FK, Nahrendorf M. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. Science 2013;339:161–166.

30 Frangogiannis NG. The inflammatory response in myocardial injury, repair, and remodelling. Nat Rev Cardiol 2014;11:255–265.

31 Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. Cardiovasc Res 2002;53:31–47.

32 Toldo S, Mezzaroma E, Mauro AG et al. The inflammasome in myocardial injury and cardiac remodeling. Antioxid Redox Signal 2015; 22:1146–1161.

33 Dobaczewski M, Gonzalez-Quesada C, Frangogiannis NG. The extracellular matrix as a modulator of the inflammatory and reparative response following myocardial infarction. J Mol Cell Cardiol 2010;48:504–511.

34 Webber J, Meran S, Steadman R et al. Hyaluronan orchestrates transforming growth factor-beta1-dependent maintenance of myofibroblast phenotype. J Biol Chem 2009;284: 9083–9092.

35 Hattori N, Carrino DA, Lauer ME et al. Pericellular versican regulates the fibroblast-myofibroblast transition: A role for ADAMTS5 protease-mediated proteolysis. J Biol Chem 2011;286:34298–34310.

36 van den Borne SW, Diez J, Blankesteijn WM et al. Myocardial remodeling after infarction: The role of myofibroblasts. Nat Rev Cardiol 2010;7:30–37.

37 Tomasek JJ, Gabbiani G, Hinz B et al. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 2002;3:349–363.

38 Thompson SA, Copeland CR, Reich DH et al. Mechanical coupling between myofibroblasts and cardiomyocytes slows electric conduction in fibrotic cell monolayers. Circulation 2011;123:2083–2093.

39 Rosker C, Salvarani N, Schmutz S et al. Abolishing myofibroblast arrhythmogeneicity by pharmacological ablation of alpha-smooth muscle actin containing stress fibers. Circ Res 2011;109:1120–1131.

40 Pellman J, Zhang J, Sheikh F. Myocyte-fibroblast communication in cardiac fibrosis and arrhythmias: Mechanisms and model systems. J Mol Cell Cardiol 2016;94:22–31.

41 Askar SF, Bingen BO, Swildens J et al. Connexin43 silencing in myofibroblasts prevents arrhythmias in myocardial cultures: Role of maximal diastolic potential. Cardiovasc Res 2012;93:434–444.

42 Askar SF, Ramkisoensing AA, Schalij MJ et al. Antiproliferative treatment of myofibroblasts prevents arrhythmias in vitro by limiting myofibroblast-induced depolarization. Cardiovasc Res 2011;90:295–304.

43 Thompson SA, Burridge PW, Lipke EA et al. Engraftment of human embryonic stem cell derived cardiomyocytes improves conduction in an arrhythmogenic in vitro model. J Mol Cell Cardiol 2012;53:15–23.

44 Kawatou M, Masumoto H, Fukushima H et al. Modelling Torsade de Pointes arrhythmias in vitro in 3D human iPS cell-engineered heart tissue. Nat Commun 2017;8:1078.

45 Askar SF, Ramkisoensing AA, Atsma DE et al. Engraftment patterns of human adult mesenchymal stem cells expose electrotonic and paracrine proarrhythmic mechanisms in myocardial cell cultures. Circ Arrhythm Electrophysiol 2013;6:380–391.

46 McDowell KS, Arevalo HJ, Maleckar MM et al. Susceptibility to arrhythmia in the infarcted heart depends on myofibroblast density. Biophys J 2011;101:1307–1315.

47 Karagueuzian HS. Targeting cardiac fibrosis: A new frontier in antiarrhythmic therapy? Am J Cardiovasc Dis 2011;1:101–109.

48 Liu YW, Chen B, Yang X et al. Human embryonic stem cell-derived cardiomyocytes restore function in infarcted hearts of non-human primates. Nat Biotechnol 2018;36:597–605.

49 Chong JJ, Yang X, Don CW et al. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. Nature 2014;273:277.

50 Menasche P. Stem cell therapy for heart failure: Are arrhythmias a real safety concern? Circulation 2009;119:2735–2740.

51 Hynes RO, Naba A. Overview of the matrisome—An inventory of extracellular matrix constituents and functions. Cold Spring Harb Perspect Biol 2012;4:a004903.

52 Collier P, Watson CJ, van Es MH et al. Getting to the heart of cardiac remodeling; how collagen subtypes may contribute to phenotype. J Mol Cell Cardiol 2012;52:148–153.

53 Talman V, Ruskoaho H. Cardiac fibrosis in myocardial infarction-from repair and remodeling to regeneration. Cell Tissue Res 2016;365:563–581.

54 Gonzalez-Santamaria J, Villalba M, Busnadiego O et al. Matrix cross-linking lysyl oxidases are induced in response to myocardial infarction and promote cardiac dysfunction. Cardiovasc Res 2016;109:67–78.

55 Lopez B, Gonzalez A, Querejeta R et al. Alterations in the pattern of collagen deposition may contribute to the deterioration of systolic function in hypertensive patients with heart failure. J Am Coll Cardiol 2006;48:89–96.

56 Jacot JG, Martin JC, Hunt DL. Mechanobiology of cardiomyocyte development. J Biomech 2010;43:93–98.

57 Roberts MA, Tran D, Coulombe KL et al. Stromal cells in dense collagen promote cardiomyocyte and microvascular patterning in engineered human heart tissue. Tissue Eng Part A 2016;22:633–644.

58 Engler AJ, Carag-Krieger C, Johnson CP et al. Embryonic cardiomyocytes beat best on a matrix with heart-like elasticity: Scar-like rigidity inhibits beating. J Cell Sci 2008;121:3794–3802.

59 Lee S, Serpooshan V, Tong X et al. Contractile force generation by 3D hiPSC-derived cardiac tissues is enhanced by rapid establishment of cellular interconnection in matrix with muscle-mimicking stiffness. Biomaterials 2017;131:111–120.

60 Forte G, Pagliari S, Ebara M et al. Substrate stiffness modulates gene expression and phenotype in neonatal cardiomyocytes in vitro. Tissue Eng Part A 2012;18:1837–1848.

61 Pagano F, Angelini F, Castaldo C et al. Normal versus pathological cardiac fibroblast-derived extracellular matrix differentially modulates cardiosphere-derived cell paracrine properties and commitment. Stem Cells Int 2017;2017:7396462.

62 Castaldo C, Di Meglio F, Miraglia R et al. Cardiac fibroblast-derived extracellular matrix (biomatrix) as a model for the studies of cardiac primitive cell biological properties in normal and pathological adult human heart. Biomed Res Int 2013;2013:352370.

63 Zhang J, Klos M, Wilson GF et al. Extracellular matrix promotes highly efficient cardiac differentiation of human pluripotent stem cells: The matrix sandwich method. Circ Res 2012;111:1125–1136.

64 Guilak F, Cohen DM, Estes BT et al. Control of stem cell fate by physical interactions with the extracellular matrix. Cell Stem Cell 2009;5:17–26.

65 Humphrey JD, Dufresne ER, Schwartz MA. Mechanotransduction and extracellular matrix homeostasis. Nat Rev Mol Cell Biol 2014;15:802–812.

66 Watt FM, Huck WTS. Role of the extracellular matrix in regulating stem cell fate. Nat Rev Mol Cell Biol 2013;14:467–473.

67 van der Flier A, Sonnenberg A. Function and interactions of integrins. Cell Tissue Res 2001;305:285–298.

68 Valencik ML, Zhang D, Punske B et al. Integrin activation in the heart: A link between electrical and contractile dysfunction? Circ Res 2006;99:1403–1410.

69 Zemljic-Harpf A, Manso AM, Ross RS. Vinculin and talin: Focus on the myocardium. J Investig Med 2009;57:849–855.

70 Tan PM, Buchholz KS, Omens JH et al. Predictive model identifies key network regulators of cardiomyocyte mechano-signaling. PLoS Comput Biol 2017;13:e1005854.

71 Rodriguez AG, Han SJ, Regnier M et al. Substrate stiffness increases twitch power of neonatal cardiomyocytes in correlation with changes in myofibril structure and intracellular calcium. Biophys J 2011;101:2455–2464.

72 Pandey P, Hawkes W, Hu J et al. Cardiomyocytes sense matrix rigidity through a combination of muscle and non-muscle myosin contractions. Dev Cell 2018;44:326.e323–336.e323.

73 Vicente-Manzanares M, Ma X, Adelstein RS et al. Non-muscle myosin II takes centre stage in cell adhesion and migration. Nat Rev Mol Cell Biol 2009;10:778–790.

74 Krishnan L, Hoying JB, Nguyen H et al. Interaction of angiogenic microvessels with the extracellular matrix. Am J Physiol Heart Circ Physiol 2007;293:H3650–H3658.

75 Shen J, Xie Y, Liu Z. Increased myocardial stiffness activates cardiac microvascular endothelial cell via VEGF paracrine signaling in cardiac hypertrophy. J Mol Cell Cardiol 2018;122:140–151.

76 Rouwkema J, Khademhosseini A. Vascularization and angiogenesis in tissue engineering: Beyond creating static networks. Trends Biotechnol 2016;34:733–745.

77 Virag JI, Murry CE. Myofibroblast and endothelial cell proliferation during murine myocardial infarct repair. Am J Pathol 2003;163:2433–2440.

78 Wang B, Ansari R, Sun Y et al. The scar neovasculature after myocardial infarction in rats. Am J Physiol Heart Circ Physiol 2005;289:H108–H113.

79 Ferrari G, Cook BD, Terushkin V et al. Transforming growth factor-beta 1 (TGF-beta1) induces angiogenesis through vascular endothelial growth factor (VEGF)-mediated apoptosis. J Cell Physiol 2009;219:449–458.

80 Durand MJ, Ait-Aissa K, Gutterman DD. Regenerative angiogenesis: Quality over quantity. Circ Res 2017;120:1379–1380.

81 Zhang B, Montgomery M, Chamberlain MD et al. Biodegradable scaffold with built-in vasculature for organ-on-a-chip engineering and direct surgical anastomosis. Nat Mater 2016;15:669–678.

82 Laschke MW, Menger MD. Vascularization in tissue engineering: Angiogenesis versus inosculation. European surgical research. Europaische Chirurgische Forschung Recherches Chirurgicales Europeennes 2012;48:85–92.

83 Mirabella T, MacArthur JW, Cheng D et al. 3D-printed vascular networks direct therapeutic angiogenesis in ischaemia. Nat Biomed Eng 2017;1:0083.

84 Weinberger F, Breckwoldt K, Pecha S et al. Cardiac repair in Guinea pigs with human engineered heart tissue from induced pluripotent stem cells. Sci Transl Med 2016;8:363ra148.

85 Vazir A, Fox K, Westaby J et al. Can we remove scar and fibrosis from adult human myocardium? Eur Heart J 2019;40:960–966.

86 Menasche P. Cell therapy trials for heart regeneration—Lessons learned and future directions. Nat Rev Cardiol 2018;15:659–671.

87 Quinn TA, Camelliti P, Rog-Zielinska EA et al. Electrotonic coupling of excitable and nonexcitable cells in the heart revealed by optogenetics. Proc Natl Acad Sci USA 2016;113:14852–14857.

88 Vasquez C, Mohandas P, Louie KL et al. Enhanced fibroblast-myocyte interactions in response to cardiac injury. Circ Res 2010;107: 1011–1020.

89 Figeac F, Lesault PF, Le Coz O et al. Nanotubular crosstalk with distressed cardiomyocytes stimulates the paracrine repair function of mesenchymal stem cells. Stem Cells 2014;32: 216–230.

90 Gourdie RG, Dimmeler S, Kohl P. Novel therapeutic strategies targeting fibroblasts and fibrosis in heart disease. Nat Rev Drug Discov 2016;15:620–638.

91 Park A, Barrera-Ramirez J, Ranasinghe I et al. Use of statins to augment progenitor cell function in preclinical and clinical studies of regenerative therapy: A systematic review. Stem Cell Rev 2016;12:327–339.

92 Cai A, Qiu R, Li L et al. Atorvastatin treatment of rats with ischemia-reperfusion injury improves adipose-derived mesenchymal stem cell migration and survival via the SDF-1alpha/CXCR-4 axis. PLoS One 2013;8:e79100.

93 Zhang Z, Li S, Cui M et al. Rosuvastatin enhances the therapeutic efficacy of adipose-derived mesenchymal stem cells for myocardial infarction via PI3K/Akt and MEK/ERK pathways. Basic Res Cardiol 2013;108:333.

94 Yang YJ, Qian HY, Huang J et al. Combined therapy with simvastatin and bone marrow-derived mesenchymal stem cells increases benefits in infarcted swine hearts. Arterioscler Thromb Vasc Biol 2009;29:2076–2082.

95 Shishehbor MH, Brennan ML, Aviles RJ et al. Statins promote potent systemic antioxidant effects through specific inflammatory pathways. Circulation 2003;108:426–431.

96 Leenders GJ, Smeets MB, van den Boomen M et al. Statins promote cardiac infarct healing by modulating endothelial barrier function revealed by contrast-enhanced magnetic resonance imaging. Arterioscler Thromb Vasc Biol 2018;38: 186–194.

97 Shiroshita-Takeshita A, Brundel BJ, Burstein B et al. Effects of simvastatin on the development of the atrial fibrillation substrate in dogs with congestive heart failure. Cardiovasc Res 2007;74: 75–84.

98 Yang C, Madonna R, Li Y et al. Simvastatin-enhanced expression of promyogenic nuclear factors and cardiomyogenesis of murine embryonic stem cells. Vascul Pharmacol 2014; 60:8–16.

99 Izadpanah R, Schachtele DJ, Pfnur AB et al. The impact of statins on biological characteristics of stem cells provides a novel explanation for their pleiotropic beneficial and adverse clinical effects. Am J Physiol Cell Physiol 2015; 309:C522–C531.

100 Carson RA, Rudine AC, Tally SJ et al. Statins impact primary embryonic mouse neural stem cell survival, cell death, and fate through distinct mechanisms. PLoS One 2018; 13:e0196387.

101 Samuel CS, Cendrawan S, Gao XM et al. Relaxin remodels fibrotic healing following myocardial infarction. Lab Invest 2011;91:675–690.

102 Swaney JS, Roth DM, Olson ER et al. Inhibition of cardiac myofibroblast formation and collagen synthesis by activation and overexpression of adenylyl cyclase. Proc Natl Acad Sci USA 2005;102:437–442.

103 Reinecke H, Robey TE, Mignone JL et al. Lack of thrombospondin-2 reduces fibrosis and increases vascularity around cardiac cell grafts. Cardiovasc Pathol 2013;22:91–95.

104 Samanta S, Balasubramanian S, Rajasingh S et al. MicroRNA: A new therapeutic strategy for cardiovascular diseases. Trends Cardiovasc Med 2016;26:407–419.

105 van Rooij E, Sutherland LB, Thatcher JE et al. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc Natl Acad Sci USA 2008; 105:13027–13032.

106 Tong Z, Solanki A, Hamilos A et al. Application of biomaterials to advance induced pluripotent stem cell research and therapy. EMBO J 2015;34:987–1008.

107 Rane AA, Christman KL. Biomaterials for the treatment of myocardial infarction: A 5-year update. J Am Coll Cardiol 2011;58: 2615–2629.

108 Li J, Minami I, Shiozaki M et al. Human pluripotent stem cell-derived cardiac tissue-like

constructs for repairing the infarcted myocardium. Stem Cell Rep 2017;9:1546–1559.

109 Mannhardt I, Breckwoldt K, Letuffe-Breniere D et al. Human engineered heart tissue: Analysis of contractile force. Stem Cell Rep 2016;7:29–42.

110 Joanne P, Kitsara M, Boitard SE et al. Nanofibrous clinical-grade collagen scaffolds seeded with human cardiomyocytes induces cardiac remodeling in dilated cardiomyopathy. Biomaterials 2015;80:157–168.

111 Ungerleider JL, Christman KL. Concise review: Injectable biomaterials for the treatment of myocardial infarction and peripheral artery disease: Translational challenges and progress. Stem Cells Translational Medicine 2014;3:1090–1099.

112 Le LV, Mohindra P, Fang Q et al. Injectable hyaluronic acid based microrods provide local micromechanical and biochemical cues to attenuate cardiac fibrosis after myocardial infarction. Biomaterials 2018;169:11–21.

113 Pinney JR, Du KT, Ayala P et al. Discrete microstructural cues for the attenuation of fibrosis following myocardial infarction. Biomaterials 2014;35:8820–8828.

114 Jha AK, Tharp KM, Browne S et al. Matrix metalloproteinase-13 mediated degradation of hyaluronic acid-based matrices orchestrates stem cell engraftment through vascular integration. Biomaterials 2016;89:136–147.

115 Shen D, Tang J, Hensley MT et al. Effects of matrix metalloproteinases on the performance of platelet fibrin gel spiked with cardiac stem cells in heart repair. Stem Cells Translational Medicine 2016; 5:793–803.

116 Sonnenberg SB, Rane AA, Liu CJ et al. Delivery of an engineered HGF fragment in an extracellular matrix-derived hydrogel prevents negative LV remodeling post-myocardial infarction. Biomaterials 2015;45:56–63.

117 Park DSJ, Mewhort HEM, Teng G et al. Heparin augmentation enhances bioactive properties of acellular extracellular matrix scaffold. Tissue Eng Part A 2018;24:128–134.

118 Fan Z, Guan J. Antifibrotic therapies to control cardiac fibrosis. Biomater Res 2016; 20:13.