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 anti-fibrotic 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.

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 anti-fibrotic 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
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 anti-fibrotic 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.

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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 automatocity 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-contraction tissue environment [52]. Collagen turnover in the maturation phase is maintained by the remaining myofibroblasts, and pro-migratory, soft collagen (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 cardiospheres-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 matrixtell 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 contractile [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 inosculuation 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
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 approaches provide beneficial effects, they may not be sufficient to fully reverse fibrosis or restore myocardial function. To address these limitations, researchers have explored the use of pharmacological agents that specifically target the fibrotic process. These agents include anti-fibrotic drugs such as angiotensin-converting enzyme inhibitors and statins, as well as other classes of medications such as peroxisome proliferator-activated receptor (PPAR) agonists and mTOR inhibitors. These agents modulate cellular pathways involved in fibrosis, such as the transforming growth factor-beta (TGF-β) signaling pathway, which plays a central role in fibrotic remodeling. By inhibiting TGF-β, these agents can reduce fibroblast activation and collagen production, leading to a reduction in fibrosis.

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.

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).
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

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).
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 adenyl 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 poly(lactic-co-glycolic 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 antibiogenic 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**

J.L.: manuscript writing, final approval of the manuscript; W.H., L.J., C.P., X.L.: manuscript editing; Y.W.: manuscript writing, financial support, final approval of manuscript.

**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.
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