

Mending a Faltering Heart

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Abstract: More people die every year from ischemic heart disease than any other disease. Because the human heart lacks sufficient ability to replenish the damaged cardiac muscles, extensive research has been devoted toward understanding the homeostatic and regenerative potential of the heart and to develop regenerative therapies for heart disease. Here, we discuss recent advances in the understanding of mechanisms governing heart growth during homeostasis or injury, including those from observational studies in humans and experimental research in animal models of cardiac regeneration. We also discuss how progress in stem cell biology and cellular reprogramming has enabled exciting new strategies for cardiac regeneration. (*Circ Res.* 2016;118:344-351. DOI: 10.1161/CIRCRESAHA.115.306820.)

Key Words: cellular reprogramming ■ induced pluripotent stem cells ■ myocardial infarction ■ myocardial ischemia ■ myocytes, cardiac

According to statistics from the World Health Organization, ischemic heart disease is the number one cause of death in the world population. It amounted to 7.4 million deaths in 2012 and has since shown a depressing upward trend in the number of lives claimed per year (<http://www.who.int/mediacentre/factsheets/fs310/en/>). Ischemic heart disease is caused by narrowing of coronary heart arteries, which restricts blood supply to ventricular muscles. A severe ischemia event will result in acute myocardial infarction (MI), characterized by massive loss of cardiomyocytes. This lost myocardial tissue cannot be regenerated because of a lack of innate ability of the human heart to replenish large numbers of cardiomyocytes and is replaced by fibrotic scar tissues instead. As a result, the infarct region is electrically uncoupled from the rest of the myocardium, leading to the loss of contractile function, pathological remodeling of the ventricular walls, and eventually heart failure. Current therapies are designed to preserve remaining cardiomyocytes and to reduce the morbidity and mortality associated with the pathological aftermath of MI. However, they flunk the heart of the mission—regeneration of lost cardiomyocytes. Here, we focus the discussion on regeneration of cardiomyocytes and point the readers on other important cardiac cell types to excellent reviews.^{1,2}

Proliferation Potential of Cardiomyocytes

An adult human left ventricle has several billion cardiomyocytes.³ They often become binucleated,⁴ contain elaborated contractile arrays called sarcomeres, and form a biomechanically aligned and electrically connected contractile unity.⁵ These features of mature cardiomyocytes have spawned a long-held paradigm that the myocardium is a terminally differentiated postmitotic tissue and that postnatal cardiomyocytes lack

proliferation capacity and grow by hypertrophy. However, this view leads to peculiar conclusions, for example, that any cardiomyocyte loss after birth will cause cardiac muscle mass to shrink and that surviving cardiomyocytes have to perform billions of contractions throughout the lifespan of the individual. This paradigm has been conclusively overturned by recent evidence from monitoring the pulse labeling of human cardiomyocyte DNA by ¹⁴C generated in the Cold War era nuclear tests⁶ and from measuring DNA synthesis using multi-isotope imaging mass spectrometry in genetically labeled mouse cardiomyocytes.⁷ A key issue on the topic of cardiomyocyte turnover is to accurately differentiate cell division from cell cycle activity that may also lead to polyploidy and multinucleation. The mouse multi-isotope imaging mass spectrometry study combined non-radioactive stable isotope detection, fluorescence in situ hybridization, and imaging to resolve this issue. These studies show that mammalian cardiomyocytes renew at a low rate ($\approx 1\%$ annually) under homeostasis, and this rate increases by roughly 4-fold (based on multi-isotope imaging mass spectrometry and imaging evidence in mouse⁷) during injury but declines with age. It is worth noting that the annual renewal values obtained by these studies matched well with the extrapolated value from a classical study performed by Soonpaa and Field in 1997.⁸

Interestingly, a recent study reported a burst of proliferation during preadolescence mouse development (postnatal day 15) that increased cardiomyocyte numbers by $\approx 40\%$.⁹ This intense cardiomyocyte proliferation seemed to be driven by an upsurge of thyroid hormone, an endogenous transcriptional activator of postnatal growth, which in turn activated the IGF-1 (insulin-like growth factor 1)/IGF-1R (IGF-1 receptor)/AKT (also known as protein kinase B, or PKB) pathway. This

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Nonstandard Abbreviations and Acronyms

CPC	cardiac progenitor cell
hESC	human embryonic stem cell
iPSC	induced pluripotent stem cell
MI	myocardial infarction

argues that the proliferation potential of postnatal mammalian cardiomyocyte may be greater than previously appreciated and, more importantly, it may be augmented by specific signals. If confirmed in humans, this phenomenon has important therapeutic implications.

Where do the newly born cardiomyocytes originate? During development, cardiomyocytes along with other major cardiac cell types, including conduction system cells, endothelial cells, smooth muscle cells, and cardiac fibroblasts, are derived from multipotent cardiac progenitor cells (CPCs) in the embryonic heart fields. The CPCs of the first heart field, marked by HCN4 (hyperpolarization-activated cyclic nucleotide-gated channel 4), contribute to cardiomyocytes of the left ventricle, areas of atria, and the conduction system in lineage-tracing studies.^{10,11} The CPCs of the second heart field, marked by the LIM/homeodomain transcription factor ISL1 also give rise to cardiomyocytes, conductive cells, and other cardiac cell types.^{12–14} The epicardium, a single layer of cells enclosing the heart, is also a source of CPC during embryogenesis. The epicardium-derived progenitor cells, marked by WT1 and TBX18, have been reported to make a substantial contribution to cardiomyocytes in the ventricular septum and the atrial and ventricular walls, as well as to smooth muscle cells and cardiac fibroblasts,^{15,16} although recent evidence indicates that more refined lineage-tracking schemes may be necessary to conclusively determine the epicardial origin of cardiomyocytes.¹⁷ Unlike the embryonic CPCs in the first and second heart fields, epicardium-derived progenitor cells are present in the adult heart. Therefore, strategies to activate these potential endogenous progenitors of cardiomyocytes could bring significant therapeutic benefits.

In the adult heart, multiple types of stem/progenitor-like cells have been reported to contribute to newly generated cardiomyocytes during homeostasis or injury repair. These can be broadly divided into the noncardiac resident type that includes bone marrow-derived cells and the cardiac resident type that includes c-Kit⁺ CPCs, Sca-1⁺ CPCs, side population cells, and epicardium-derived progenitor cells. The origin and lineage potential of these putative adult CPCs and the clinical trials and controversies associated with these cells have been discussed in detail by several excellent reviews.^{18–21} The prevailing view is that these adult CPCs do not contribute to the cardiomyocyte number at physiologically relevant levels during cardiac homeostasis, although their cardiomyogenic potential can be boosted under certain conditions,^{22,23} and that their cardioprotective properties observed in preclinical studies and clinical trials (reviewed in Ref 21) are due to paracrine effects.

In contrast to the controversy shrouding adult CPCs, there is compelling evidence supporting pre-existing cardiomyocytes as the predominant source of cardiomyocyte renewal under homeostasis. A study in mice tracked stable isotope (¹⁵N)

labeling of genetically labeled cardiomyocytes and showed that the birth of new cardiomyocytes occurs infrequently through division of pre-existing cardiomyocytes during normal aging, and the speed of cardiomyocyte renewal is accelerated by MI.⁷ Another study also concluded that the α -myosin heavy chain positive cardiomyocytes are the cells of origin in postnatal cardiomyogenesis by analyzing mosaic clones of cardiomyocytes generated by interchromosomal recombination.²⁴ However, the authors of the latter study did not observe any increase in cardiomyocyte proliferation after MI, contradicting previous findings. Whether this is because of differences in mouse models, injury types, or labeling efficiency remains an open question.

A recent study examined cardiomyocyte proliferation in heart tissues of young humans (age, 0–20) and adults (age, 21–59) using image-based assays. Cardiomyocyte mitosis was detectable throughout life, which is consistent with the ¹⁴C bomb-dating study. Cardiomyocyte division rate was the highest in infants and decreased to low levels by age 20. There was a surprising 3.4-fold increase in the cardiomyocyte number from age 1 to 20.²⁵ These findings suggest that young humans may be able to regenerate the myocardium. In contrast, cardiomyocyte cytokinesis was not detectable beyond age 20. Interestingly, there was no increase in binucleation but rather an increase of nuclear ploidy with age. The recalcitrance of adult cardiomyocytes to go through cytokinesis may have multiple causes, such as physical hindrance by sarcomeres,²⁶ lack of hormonal or neural signals controlling postnatal growth,^{5,27} and changes in epigenetic pathways.²⁸ As the body of evidence supporting cardiomyocytes as the most important cellular source of heart regeneration grows, it has become critically important to unlock the mechanisms that control mitosis and cytokinesis of adult cardiomyocytes and identify strategies to enhance cardiomyocyte regeneration.

Animal Models of Cardiac Regeneration

Animals that can naturally regenerate their heart after injury offer a window to peer into the process of cardiomyocyte regeneration. Lower animals, such as newts and zebrafish, can fully regenerate their heart after resection of $\approx 20\%$ apical myocardium.^{29–31} In the case of zebrafish, other injury models have been used to study the regeneration process. These include cryoinjury of $\approx 20\%$ of the ventricular wall^{32–34} and genetic ablation, in which $\leq 60\%$ of cardiomyocytes are killed by cardiomyocyte-specific expression of diphtheria toxin A driven by a cardiac myosin light chain 2 (*cmlc2*) promoter and tamoxifen-Cre.³⁵ Little scar formation was observed in the mechanical and genetic injury models. A fibrotic scar formed initially after cryoinjury but was gradually replaced with regenerated tissue, resulting in a more protracted yet still scarless recovery.³⁶ In contrast, the adult mammalian heart forms fibrotic scars after injury without regenerating the lost myocardium. Surprisingly, recent studies suggest that much like adult zebrafish, 1-day-old neonatal mouse can regenerate its heart after various injuries, including amputation,³⁷ ischemic MI,³⁸ and cryoinjury.^{39,40} Unlike the adult zebrafish heart whose regenerative capacity does not decrease with age,⁴¹ the regenerative ability of the neonatal murine heart is lost by postnatal day 7. Another important difference worth

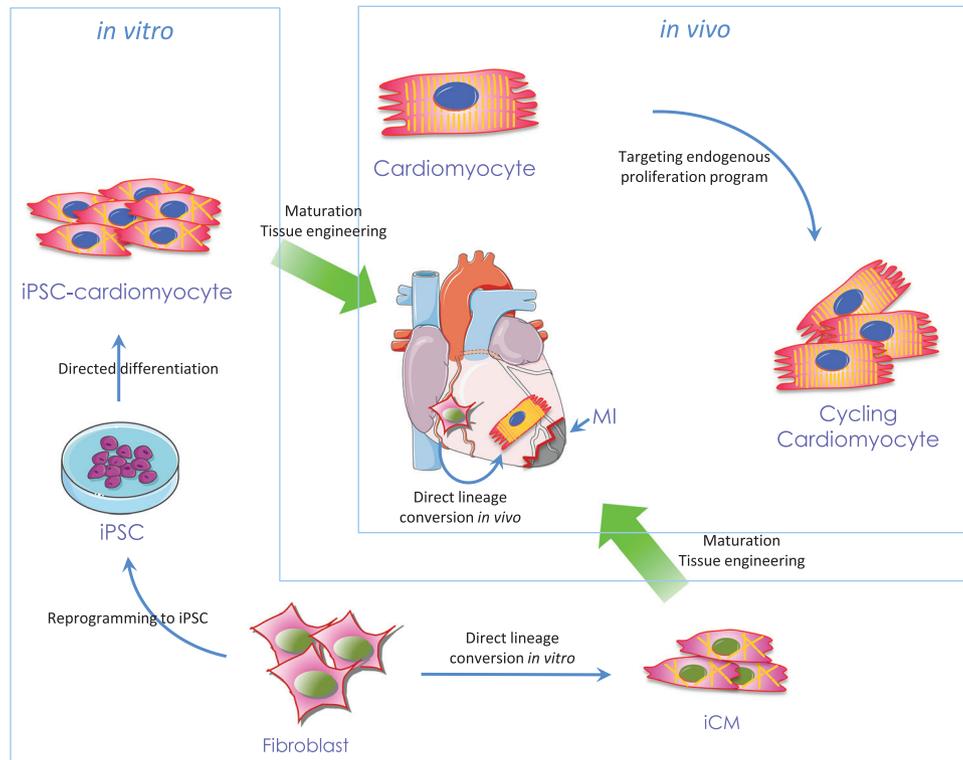


Figure. Recent strategies for cardiomyocyte regeneration. Cardiomyocytes can be induced to re-enter the cell cycle through modulation of endogenous cardiomyocyte proliferation programs. Reprogramming to induced pluripotent stem cell (iPSCs) followed by directed differentiation could provide a large number of immature cardiomyocytes. Other noncardiomyocyte cells, such as fibroblasts, can be directly converted to induced cardiomyocytes (iCMs). These *in vitro* derived cardiomyocytes may be further matured using tissue engineering technologies. *In situ* conversion of cardiac fibroblasts also represents a promising strategy for heart repair. MI indicates myocardial infarction.

noting is that the murine heart is still growing during the first few days of neonatal life and contains actively dividing cardiomyocytes, whereas the adult zebrafish heart has reached homeostasis and is largely postmitotic. Thus, it could be argued that the regenerative response mounted by the neonatal murine heart is a form of compensatory growth or at least benefits from the underlying developmental cardiomyocyte growth. Nonetheless, the transient neonatal cardiac regeneration in mice, together with the surprisingly large increase in the cardiomyocyte number during childhood and adolescence in humans, should fuel optimism in rejuvenating the regenerative potential of the adult human heart therapeutically.

In zebrafish, the regeneration process is initiated by reactivation of developmental programs in the epicardium, myocardium, and endocardium.⁴² Activation of epicardium and endocardium provides paracrine factors, including retinoic acid and CXCL12a, that are essential for cardiomyocyte proliferation and migration.^{43,44} Epicardial cells also contribute to neovascularization of the regenerating tissue through epithelial-to-mesenchymal transition, where the fibroblast growth factor signaling and platelet-derived growth factor signaling are important.^{45,46} Genetic fate-mapping experiments have conclusively shown that the cellular source of the regenerated myocardium is derived from pre-existing cardiomyocytes that have undergone dedifferentiation and subsequent proliferation.^{47,48} Dedifferentiated cardiomyocytes re-express the embryonic cardiogenesis gene *Gata4*, disassemble their sarcomeric structures, become detached from one another,

and upregulate the cell cycle regulators. The determination of the dedifferentiation process in fate-mapped pre-existing cardiomyocytes, as evidenced by sarcomere disassembly and gradual loss of mature cardiomyocyte morphology, argues against differentiated cardiac progenitors as the source of new cardiomyocytes. Once cell division is completed, regenerated cardiomyocytes undergo a maturation step and reintegrate into the myocardium, thereby restoring cardiac function.

The process of heart regeneration in neonatal murine is reminiscent of that in zebrafish.^{37,38,40} Lineage-tracing and histological studies confirmed that the regenerated cardiomyocytes come from pre-existing cardiomyocytes through cell division. In addition, Porrello et al³⁸ reported a robust angiogenic response as part of the regeneration process. There is evidence that *c-Kit*⁺ CPCs are induced to expand after injury and could contribute to both angiogenesis and cardiogenesis in the neonatal heart but not in the adult heart.³⁹

Why can't the adult mammalian heart regenerate itself? Many theories have been put forward to answer this. It has been postulated that cytokinesis is blocked in adult cardiomyocytes because of binucleation. Consistent with this idea, zebrafish cardiomyocytes are mostly mononuclear, which may help maintain their proliferative capacity during regeneration.⁴⁹ The adult mammalian heart contains binucleated cardiomyocytes, although the degree of binucleation varies among species.⁵⁰ For example, most adult murine cardiomyocytes are binucleated, whereas binucleation of human cardiomyocyte remains at a constant low level throughout life.²⁵ It has been shown

that in vivo proliferating cardiomyocytes reside primarily in the mononucleated fraction.^{25,51} Recent observation during the burst of cardiomyocyte proliferation in a preadolescence mouse suggests the possibility of cytokinesis in the binucleated population.⁹ The process of binucleation itself is under tight control by regulators of cell cycle and cytokinesis, as reviewed.⁵² Future quantitative analyses are required to clarify the relationship between the nuclearity, cell cycle status, and cell division of cardiomyocyte. To this end, a recently reported transgenic system composed of an α -myosin heavy chain positive:H2B-mCherry transgene and a CAG (a strong synthetic promoter)–eGFP (enhanced green fluorescent protein)–anillin transgene could be useful for unequivocal identification of cardiomyocyte nuclei and analysis of its cell cycle.⁵³

Compared with zebrafish hearts, mammalian hearts are of a more complex design in structure (4-chambered versus 2-chambered), tissue organization (fibroblasts account for most of the mammalian heart mass but are rare in the fish heart), mechanical properties (higher blood pressure in mammals), and electric properties to handle the higher metabolic demand of mammals. One may speculate that in such complex hearts, the large-scale dedifferentiation and electric decoupling of cardiomyocytes as seen during zebrafish heart regeneration may not be compatible with organismal survival (because of lethal arrhythmia or reduced ability to evade predation). Notwithstanding the uncertainty about the proximate causes of this lack of regenerative ability, the ultimate cause may be that evolution has favored the solution of maintaining cardiac health through diet and physical activity over that of the costly regeneration. However, one should not be dissuaded from going back in evolution to borrow strategies to enhance human heart regeneration.

Factors that regulate the evolutionarily conserved regenerative program have been under intensive investigation. Cardiomyocyte renewal can be promoted by inhibiting negative regulators of proliferation, such as the Hippo signaling pathway⁵⁴ and the p38 mitogen-activated protein kinase pathway,⁵⁵ or activating the endogenous cardiomyocyte proliferation program through neuregulin 1 signaling,⁵¹ hypoxia and cell cycle regulators polo-like kinase 1 (*plk1*), and mitotic checkpoint kinase *mps1*.^{30,56}

Micro-RNAs (miRNAs) can also coordinately regulate cardiomyogenic programs. They have been shown to be attractive therapeutic targets for heart disease.⁵⁷ A screen of miRNAs identified miR-590 and miR-199a as positive regulators of cardiomyocyte proliferation,⁵⁸ whereas miR-133 and miR15 were found to inhibit cardiomyocyte proliferation.^{38,59} Interestingly, a pluripotency-associated miRNA cluster—miR302-367—has been shown to promote cardiomyocyte proliferation, reduce scar formation, and improve cardiac function.⁶⁰ Expression of miR302-367 reactivated cell cycle through repression of the Hippo signaling pathway. The pro-myogenic effect may also due to the induction of dedifferentiation by miR302-367, which is consistent with its role in somatic cell reprogramming.⁶¹ Our group recently identified miR-99/100 and Let-7a/c as critical regulators of zebrafish heart regeneration by systematic screening of genes differentially regulated during the process.²⁸ These miRs are highly

expressed in the uninjured heart but undergo rapid downregulation during injury. We further identified *fnb* (β -subunit of farnesyl transferase) and *Smarca5* (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily a, member 5) as downstream targets of miR-99/100 and as important regulators of cardiomyocyte dedifferentiation. The same miR regulatory program was also found in adult mammalian cardiomyocytes, but it failed to be activated during injury. Experimental downregulation of miR99-100 promoted a dedifferentiated phenotype in adult murine and human cardiomyocytes, best exemplified by re-expression of GATA4 and proliferation markers and disassembly of sarcomeres. More importantly, in vivo delivery of antimirRs of miR-99/100 and Let-7a/c resulted in cardiomyocyte dedifferentiation and improved heart functionality in adult murine models of MI.

In addition to cardiac cells, nerves have been shown to be required for cardiomyocyte regeneration of both zebrafish and neonatal mice. This was demonstrated by a genetic model of hypoinnervation of the heart, pharmacological inhibition of cholinergic nerve activity, and mechanical denervation.²⁷ Administration of neuregulin 1 and nerve growth factor as recombinant proteins rescued the impaired regeneration caused by denervation. However, the connection between these factors and the nerve effect was not established. As several recent studies have demonstrated the role of neuregulin 1 and its co-receptor ERBB2 in cardiomyocyte dedifferentiation and proliferation,^{62–64} it will be of great interest to understand if these factors also act separately through the nervous system. One report has challenged the role of neuregulin 1 in activation of adult cardiomyocyte DNA synthesis, suggesting that the effect may be limited to a developmental window.⁶⁴ Interestingly, the denervated heart showed a reduced expression of inflammatory and immune pathway genes after injury. The prevailing view is that the immune system and inflammatory response play both positive and negative roles in tissue repair and regeneration. Acute inflammation has been shown to be required for proper regenerative response.^{65,66} Recent evidence further shows that macrophages are required for neonatal heart regeneration.^{67,68} These studies identified a population of embryonic-derived resident cardiac macrophages that minimized inflammation and promoted cardiomyocyte proliferation and angiogenesis in the regenerating neonatal heart. In the injured adult heart, however, these cells were replaced with monocyte-derived macrophages that were proinflammatory and lacked regenerative ability.⁶⁷ Understanding the soluble factors and cell-mediated effects provided by various cellular components of the regenerating heart requires further studies.

Cardiac Regeneration by Cellular Reprogramming

Recent advances in stem cell biology have not only enabled de novo generation of cardiomyocytes in vitro but also brought forth exciting possibilities of reprogramming noncardiomyocytes into cardiomyocytes in vivo.

In 2006, Takahashi and Yamanaka⁶⁹ discovered induced pluripotent stem cells (iPSCs), thereby opening the floodgates for generating all kinds of immunocompatible cell types for cellular therapy. In vitro differentiation of iPSCs derived from patient fibroblasts can produce individually tailored

cardiomyocytes and other cardiac cell types for disease modeling, drug screen, and cell therapy. Multiple approaches have been devised to differentiate human embryonic stem cells (hESCs) and human iPSCs to cardiomyocytes.²¹ The efforts have been focused on increasing the efficiency (and thus yield) of cardiomyocyte differentiation and on specifically driving them toward the ventricular subtype. Protocol refinement requires detailed knowledge of *in vivo* cardiogenesis. For example, the identification of first heart field and second heart field markers facilitates the isolation of distinctively fated CPCs from differentiated iPSCs¹⁰ and enables specific commitment to ventricular cardiomyocytes⁷⁰ and conductive cells.¹¹ Fine-tuning key signaling pathways that orchestrate embryonic heart development (including activin/nodal, Wnt, fibroblast growth factor, Notch, and Sonic hedgehog) during differentiation have improved the yield of cardiomyocytes. A recent study achieved robust cardiomyocyte differentiation ($\leq 98\%$ pure) of hESCs and human iPSCs via differential modulation of Wnt signaling during early and late phases of differentiation,⁷¹ which mirrored the biphasic requirement of Wnt signaling in embryonic heart development.⁷² Regulated *MYC* expression can expand multipotent CPCs isolated in hiPSC/hESC differentiation cultures, thereby providing a large number of pure progenitors that can be patterned with morphogens to differentiate into pacemaker-like or ventricular-like cardiomyocytes.⁷³ Despite the improvements in yield and purity of hESC/hiPSC-derived cardiomyocytes, the field still faces a major roadblock: the immaturity of the differentiated cells. Tissue engineering techniques, such as biodegradable scaffolds⁷⁴ and 3-dimensional (3D) constructs consisting of other supporting cells,^{75,76} have shown early promise in improving electromechanical properties and engraftment.

The first clinical-scale transplantation of hESC-derived cardiomyocytes in a nonhuman primate model was published in 2014.⁷⁷ Up to 10^9 differentiated cardiomyocytes were delivered intramyocardially to the infarcted heart. The grafts remuscularized the damaged heart, received blood perfusion from host vasculature, and integrated electromechanically to host myocardium. However, the transplanted cells failed to mature completely, and nonfatal ventricular arrhythmias were observed in recipient hearts. Although this study did not provide clear evidence that transplantation of hESC-derived cardiomyocytes improves cardiac function after MI, it sets an important optimistic baseline for future investigation. It also highlights key problems, including the logistics of producing enough cardiomyocytes in an economically feasible manner, that need to be addressed before *in vitro* derived cardiomyocytes can be tested in humans.

The idea of iPSC reprogramming also reinvigorated an old concept of direct lineage conversion first described with the transcription factor MyoD a quarter of a century ago.^{78–80} Lineage conversion technologies entail converting one somatic cell type to another without reverting back to pluripotency.^{81,82} Lineage conversion has the obvious advantage of avoiding the risk of teratoma in the heart that may originate from the chance carryover of pluripotent cells.⁸³ It may also offer a shorter timeframe from biopsy to transplantable cells compared with iPSC generation followed by directed differentiation. Ieda et

al⁸⁴ first reported that 3 cardiac-specific transcription factors, *Gata4*, *Mef2c*, and *Tbx5* (collectively referred to as GMT), were sufficient to induce a cardiomyocyte-like phenotype from mouse postnatal cardiac or dermal fibroblasts.⁸⁴ The conversion process did not go through a progenitor-like state, indicating direct conversion. GMT-transduced fibroblasts also differentiated into cardiomyocyte-like cells after transplantation into the mouse heart. The induced cardiomyocytes, however, showed a low rate of maturation. A later study reported greatly varied conversion efficiency with the GMT method and stressed the importance of alternative cardiac reporters.⁸⁵ Because of differences in experimental design, further research is needed to settle the controversy. Subsequent studies identified new transcription factors,^{86,87} cardiac-enriched miRNAs,⁸⁸ and alternative delivery methods⁸⁹ that improved on the original GMT method. Cardiac conversion of human fibroblasts requires additional factors beyond GMT, and the human induced cardiomyocytes generated so far are functionally immature.^{90–93} Details of these studies have been recently reviewed.^{21,94}

The adult heart contains a large pool of fibroblasts, which could be a good target for direct lineage conversion. When retroviral vectors expressing GMT or GMT plus *Hand2*—another cardiac transcription factor—were introduced to mouse MI models, the resident cardiac fibroblasts were reprogrammed into cardiomyocyte-like cells at efficiencies not much different from those observed *in vitro*,^{86,95} although a much lower efficiency was reported by 1 group.⁸⁹ Interestingly, the *in vivo* conversion of induced cardiomyocytes seemed more complete and resulted in a more mature cardiomyocyte phenotype than *in vitro* induced cardiomyocytes. More importantly, *in vivo* cardiomyocyte conversion improved cardiac function after MI despite being an inefficient process. These encouraging results indicate that *in vivo* lineage conversion is a promising strategy for restoring heart function.

Future Prospectives

Intensive research in recent years has led to surprise findings about the homeostatic and regenerative renewal capacity of the heart. They have in turn spurred the development of a multitude of strategies to regenerate the damaged heart (Figure). Many unresolved issues stand between current strategies and future clinical therapies. It is unclear what cell type(s) will be most suitable for transplantation therapy, which is largely because of a lack of understanding of the mechanism of action of the transplanted cells. Long-term engraftment of transplanted cardiomyocytes has not been feasible, probably because of their inability to integrate mechanically and electrically. Combining stem cell technology with bioengineering methods, such as 3D artificial cardiac constructs comprising cardiomyocytes, endothelial cells and fibroblasts, has shown promise in addressing this issue.^{96–98} A recent article from the Ruiz-Lozano group showed that reconstitution of follistatin-like 1, a regenerative factor secreted by the healthy epicardium, using a bioengineered epicardial patch improved cardiac function in mouse and swine models of MI.⁹⁹ As for these cell-free approaches, the safety of the factors—be it recombinant proteins for promoting cardiomyocyte growth, miRNAs for

promoting dedifferentiation, or viral vectors for cellular reprogramming—has to be carefully monitored in vivo. To obtain preclinical data relevant to human physiology, large animal models would have to be used.

Besides tackling these issues, researchers should also be on the lookout for other potentially disruptive technologies that are on the horizon. For example, by combining targeted genome-editing technologies¹⁰⁰ and blastocyst complementation,¹⁰¹ it may be possible to generate chimeric animals from which human heart tissues or even whole organs may be harvested. Such technologies are undoubtedly fraught with ethical issues. However, technological advancement could make it possible to find ethically acceptable animal models or developmental stages from which proliferating human cardiomyocytes can be isolated for therapy. In the future, mechanistic studies of the process of heart regeneration combined with new technological developments in the areas of stem cells, bioengineering, and genome editing will be major drivers that continue to spin the flywheel that powers the field of cardiac regeneration forward.

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Disclosures

None.

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