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Abstract

Transplantations of various stem cells or their progeny have repeatedly improved cardiac performance in animal models of myocardial injury, however, the benefits observed in clinical trials have been generally less consistent. Some of the recognized challenges are poor engraftment of implanted cells and, in the case of human cardiomyocytes, functional immaturity and lack of

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electrical integration, leading to limited contribution to the heart's contractile activity and increased arrhythmogenic risks. Advances in tissue and genetic engineering techniques are expected to improve the survival and integration of transplanted cells, and to support structural, functional, and bioenergetic recovery of the recipient hearts. Specifically, application of a prefabricated cardiac tissue patch to prevent dilation and to improve pumping efficiency of the infarcted heart offers a promising strategy for making stem cell therapy a clinical reality.

Keywords

biocompatible materials; heart failure; myocardial infarction; myocardium; stem cells

Introduction

Although transplanted cells and engineered tissues result in improved cardiac performance when tested in animal models of myocardial injury, the benefits observed in clinical trials have generally been modest at best. Opinions regarding the optimal cell type or combination of cell types have yet to reach consensus, and only a very small proportion of the administered cells are engrafted by the native myocardium. Cellular attrition is often attributed to a lack of perfusion in the infarcted region, but the recipient's immune system may also play a role, particularly in preclinical studies with human-derived tissues or other xenogeneic transplantation experiments. Furthermore, the surviving cells rarely produce grafts of substantial size and may remain electrically isolated from the native myocardium, which would prevent the graft from contributing to the contractile activity of the heart and, more importantly, could lead to arrhythmogenic complications, which may be the primary safety concern associated with transplanted myocardial cells and tissues. Tissue engineering strategies are expected to improve engraftment of transplanted cells, as well as structural, functional, and bioenergetic recovery of the infarcted heart. These and many other topics were discussed by attendees of the National Institutes of Health 2016 Progenitor Cell Biology Consortium and Cardiovascular Tissue Engineering Symposium at the University of Alabama, Birmingham on March 28, 2016. Here, we present some of the more provocative ideas and advances that were discussed at the meeting and that may facilitate the translation of cardiac cell- and tissue-engineering therapies from the laboratory to the clinic.

Cell Types for Use in Cardiac Therapy

A wide variety of cell sources have been evaluated for repair of the ischemic myocardium in animal models, and a subset of these have undergone testing in clinical trials. A recent review by Nguyen et al. (1) summarized the clinical trials on stem cell therapy for ischemic heart diseases and heart failure from January 1, 2000 to July 2016. Table 1 adds the new clinical trials for ischemic heart diseases and heart failure published between July 27, 2016 and May 18, 2017, based on our PubMed search results, as well as clinical trials for stem cell therapy for congenital heart diseases.

Human pluripotent stem cells (hPSCs) and newer cell types, such as induced cardiac progenitor cells (iCPCs) (2,3), are especially promising for cardiac cell and tissue engineering therapy (4–6) because they can be efficiently differentiated into functional

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cardiomyocytes (CMs), endothelial cells (ECs), and smooth muscle cells (SMCs) (7–11). However, the optimal proportions of each cell type have yet to be identified, and a variety of other cell lineages (e.g., resident cardiac progenitor cells [CPCs]) (12) may be needed to maximize therapeutic effectiveness. Furthermore, mitochondria have important functions in cardiac metabolism (13) and programmed cardiomyocyte death (14), and emerging roles in cardiac differentiation were recently suggested (15,16). Specifically, CM differentiation requires the membranes of adjacent mitochondria to fuse, and fusion of the outer membranes is regulated by mitofusins 1 and 2, which direct ESC differentiation into cardiomyocytes via regulation of calcineurin and Notch signaling (16).

The importance of restoring functional cardiomyocytes for post-infarction repair is self-evident because they provide the mechanical force needed for contraction. In a rat model of myocardial infarction (MI), measurements of cardiac function and remodeling were significantly better when implanted fibrin gel-based tissue-engineered patches were created from the complete population of neonatal rat cardiac cells than when the cardiomyocytes were omitted (17). Furthermore, extracellular matrix production in tissue-engineered patches appears to increase in response to production of transforming growth factor β1 by cardiomyocytes (18,19), and cardiomyocytes are an important source of vascular endothelial growth factor in cell sheet–based engineered tissues (20). Thus, cardiomyocytes and cytokines that mediate CM–non-CM communication are crucial components of the beneficial activity induced by transplanted engineered cardiac tissues.

Other cell sources of potential utility for cardiac repair include mesenchymal stem cells (prototypically derived from bone barrow), cardiac stem cells, cardiospheres isolated from endocardial biopsies (in mice and humans), which are composed of a heterogeneous cell population, but are exceptionally proliferative, and Abcg2-expressing progenitor cells (21). When suspended in saline and transplanted into the hearts of mice after an acute infarction, Sca-1^{+}/$CD31^{-}$ cells appear to attenuate decline in cardiac function, increase myocardial neovascularization, and modestly promote cardiomyocyte differentiation from graft cells, as well as host cell proliferation (22).

Variation of interspecies responses to cell therapy is another critical issue. Commonly used preclinical models include nonhuman primates, large mammals (swine, dog, and others), and rodents. Although it might be necessary, there is neither guidance on selection of preclinical animal models nor consensus criteria on experimental design for preclinical studies thus far. Genetic background may affect the interpretation of experimental results. Therefore, comparison and validation of data collected from different species is important when translating preclinical research to clinical trials.

Although transplantation of various cell types has been reported to improve left ventricular (LV) function and structure after MI, overwhelming evidence has demonstrated that there is no significant long-term engraftment of adoptively transferred cells into the host myocardium. The persistence of beneficial effects, despite the disappearance of transplanted cells, indicates that cell therapy may act via paracrine mechanisms. In addition, the rapid clearance of cells from the host myocardium suggests that the benefits of cell therapy are limited by the poor engraftment of the cells, implying that 1 dose does not adequately test

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the efficacy of that cell product (23). However, almost all preclinical and clinical studies of cell therapy performed heretofore have based their assessment of efficacy on the outcome of 1 administration of a cell product. The problem of modest or no beneficial effects might be overcome by repeated cell doses. The rationale is that just as most pharmacological agents are ineffective when given once, but can be highly effective when given repeatedly, a cell product might be ineffective or modestly effective as a single treatment, but might be quite efficacious if given repeatedly.

On the basis of preclinical data performed in large animal models, human testing of various adult cell sources has progressed from phase I to phase III trials (24,25). From a mechanistic standpoint, cell therapy in the iterations described earlier reduces tissue fibrosis, restores tissue perfusion, has a powerful anti-inflammatory effect, and stimulates myogenesis, largely by promoting endogenous myogenesis (24). Moreover, clinical trial activity is extending into numerous cardiomyopathic disease states, including hypoplastic left heart syndrome, adriamycin-induced cardiomyopathy, and idiopathic dilated cardiomyopathy. Future efforts to apply tissue-engineering strategies in the clinical setting will emerge following appropriate preclinical testing.

Cell Engraftment

As many as 1 billion cardiomyocytes are lost during MI, and although the typical dose of transplanted cells may approach or exceed this number, just 0.1% to 10% of the cells are engrafted into the myocardium and continue to survive for more than a few weeks after transplantation (26,27). Much of this attrition can be attributed to the harsh environment in and near the region of the infarct; thus, one strategy for improving engraftment and survival of transplanted cells is to use natural or artificial biomaterials that provide a protective environment for the transplanted cells (28–30). Overall, published reports indicate that the functional benefits of the cardiac cell patch therapy critically depend upon the longer-term structural integrity of the cell patches. Maintenance of longer-term graft size remains a major challenge in preclinical trials of cell therapy.

Immunogenicity

One of the chief benefits of human induced pluripotent stem cells (hiPSCs) is that they can be generated from a patient’s own somatic cells, and consequently are not expected to provoke an immune response after transplantation. Although this approach holds appeal for avoiding pharmacological immunosuppression and associated complications, it requires a substantial time window (months) for hiPSC generation, followed by differentiation and graft formation. Thus, this autologous approach is better suited to treatment of more chronic heart failure with existing technology. However, some studies suggest that the immune tolerance of patient-specific induced pluripotent stem cell (iPSC)–derived cells may vary depending on the cells’ lineage (31–34). Questions regarding immunogenicity are often addressed by performing experiments in mice with humanized immune systems, but the currently available models have a limited lifespan and inconsistent immune response, perhaps because the animals’ endogenous immune system is eliminated with sublethal doses of radiation. Collectively, these observations suggest that translation of hiPSC-derived cell

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technology to clinical applications will likely require testing of the immunogenicity of each hiPSC-derived cell lineage in a new generation of models that can provide more consistent and reliable results.

Allograft rejection is primarily mediated by host-derived reactive T lymphocytes that recognize nonself human leukocyte antigens (HLAs) on the surface of transplanted donor cells. Humans have 2 main categories of HLAs: major histocompatibility complex (MHC) classes I and II. The Townes laboratory recently showed that surface expression of HLA class I molecules can be largely eliminated in a line of human embryonic stem cells (H9) using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) gene-editing system to knock out both alleles of the gene for β2 microglobulin, which is essential for cell-surface expression of HLA class I and stability of the peptide-binding groove. CRISPR/Cas gene editing has also been used to knock out the Class II MHC transactivator (CIITA) in human ECs, and the CIITA-knockout ECs could be transplanted into mice without producing an immune response (35). The degree of differentiation of the hiPSCs derivatives engrafted may also influence the immunogenicity of the tissue grafts. This is particularly relevant for tissue-engineering applications, because even when professional antigen-presenting cells are depleted, cell-mediated allograft rejection can still occur (36). This is perhaps because human ECs activate alloantigen-reactive memory $CD4^+$ T cells via a mechanism requiring expression of class II MHCs. Thus, gene-editing technologies may enable researchers to create “universal donor” hiPSC lines, as well as hiPSC-derived cells and engineered tissues with substantially higher rates of engraftment that can be used to treat a wider variety of patients and diseases.

Immunomodulation

As described earlier, stem cell engraftment rates and survival following transplantation are disappointingly low. Moreover, among surviving transplanted progenitor cells, the demonstrable magnitude of differentiation into functional cardiomyocytes has been variable, ranging from no evidence of cardiomyocyte differentiation to generation of small, apparently integrated cardiomyocytes (22,26,37–39). Most studies of cell therapy seek to intervene therapeutically either after MI or during chronic heart failure. The proinflammatory environment of the failing heart may also be responsible for the reported functional benefits of cardiac cell therapies. As both of these pathological scenarios exhibit heightened inflammatory activation and innate and adaptive immune cell infiltration in the myocardium (40–43), these microenvironmental factors may be important contributors to the suboptimal responses to cell therapy. For example, tumor necrosis factor (TNF), a proinflammatory cytokine elaborated by both immune cells and failing cardiomyocytes, restrains cardiomyocyte differentiation of resident cardiac stem cells, and can channel an alternate neuroadrenergic-like fate in vitro (44); these effects would be expected to diminish the reparative effects of stem cell therapy. Such findings suggest that immunomodulation of the proinflammatory microenvironment in recipients of progenitor cell therapy may be a high-yield strategy to enhance cell engraftment and cardiomyocyte differentiation. To date, such approaches have been relatively unexplored, but could include targeting specific innate immune cell populations (e.g., infiltrating and proinflammatory macrophages), specific cytokines (e.g., TNF), and/or antigen-independent T-cell responses. These methodologies

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would be complementary to the suppression of antigen-dependent MHC responses described earlier, and would ideally comprise circumscribed interventions designed to improve cell engraftment at the time of delivery and subsequent cardiomyocyte differentiation during initial repair. Interestingly, mesenchymal stem cells suppress TNF levels substantially, and this effect may therefore contribute to some of the positive effects of these cells in clinical trials (24).

Arrhythmogenesis

Injected hPSC–derived cardiomyocytes have not been associated with arrhythmias in rodents, but when the dose was scaled up for delivery to nonhuman primates (macaques), all 4 of the cell-treated animals experienced periods of premature ventricular contractions and/or ventricular tachycardia (30). These results were recently confirmed in a larger set of macaques upon allogeneic transplantation of macaque pluripotent stem cell (PSC)–derived cardiomyocytes suspended in prosurvival cocktail (45). The discrepancy between observations in rodents and macaques may have occurred because the large dose of cells administered to macaques was accompanied by a dramatic (≥10-fold) increase in tissue graft size. As the action potential passes through the myocardium, the anatomic and/or functional heterogeneity introduced by these large regions of immature, electrically-active tissue may slow down or partially block conduction, thus setting the conditions for life-threatening re-entrant arrhythmias (46). As they are electrically immature and contain sinoatrial nodal cells, hPSC-cardiomyocytes possess autonomous pacemaking activity and, consequently, are capable of ectopic beats that could further precipitate arrhythmia induction. If exogenous cells indeed engraft at much higher rate, this may result in even more severe ventricular arrhythmias. Of note, compared with human hearts, macaque hearts are much smaller and their resting rate is much higher, which raises doubts as to their suitability for predicting arrhythmogenic risks in humans. Although larger animals, such as pigs, are a better model of human heart physiology, objective assessment of human cell therapies in large animals will require adequate immunosuppression, which may be easiest to achieve in nonhuman primates.

In adult mammalian hearts, electrical propagation and myocardial contractions are coordinated primarily through the gap-junction proteins connexin 40, connexin 43 (Cx43), and connexin 45 (47), of which Cx43 is by far the most abundant. Cx43 is expressed in both atrial and ventricular myocytes (48), and deficiencies in Cx43 expression or organization have been linked to development of arrhythmias in patients with heart failure and other cardiomyopathies (47,49,50). Furthermore, previous studies (51) suggested that the risk of arrhythmogenic complications from transplanted cells may decline substantially if the cells were genetically modified to overexpress Cx43. Thus, graft-associated arrhythmogenicity may be substantially reduced by using gene-editing technologies to increase expression of gap-junction proteins in transplanted cardiomyocytes or surrounding nonmyocytes. Still, the small cell size and immature expression and distribution of ion channels and gap junctions in transplanted cardiomyocytes, as well as the isotropic architecture of the grafts, likely contributed to the occurrence of arrhythmias in macaques, despite proven host-graft Cx43 coupling (30,45). Additional experimental and computational studies are warranted to

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establish critical structural and functional properties of transplanted grafts leading to increased arrhythmia susceptibility.

Furthermore, the results from a recent study (52) suggest that intramyocardially-injected cardiac microtissue particles (consisting of ~1,000 cells/particle) suspended in prosurvival cocktail produce grafts that are electrically coupled to the native myocardium, but an epicardially-implanted engineered cardiac tissue patch does not. The combined use of genetically-encoded fluorescent calcium reporters (e.g., GCaMP) (45,53) targeted to transplanted cells and voltage-sensitive dyes with nonoverlapping emission spectra labeling host myocardial tissue will be valuable for exploring the mechanisms of arrhythmogenesis and evaluating the effectiveness of strategies for improving electromechanical integration of engineered myocardial grafts.

Myocardial Bioenergetics

The contractile activity of engineered myocardial tissue is expected to contribute directly to myocardial performance, but improvements can also evolve through the release of cytokines that promote angiogenesis, activate endogenous progenitor cells (20,54), or stimulate other beneficial paracrine pathways. Furthermore, the damage induced by an acute infarct event is exacerbated by chronic myocardial overload, dilation, and overstretching, which increases wall stress and can lead to metabolic abnormalities, such as declines in the rate of adenosine triphosphate (ATP) use or in the ratio of phosphocreatine to ATP in surrounding cardiomyocytes (55–58). These bioenergetic abnormalities were largely corrected when hiPSCs were differentiated into hiPSC-ECs and hiPSC-SMCs, and then suspended in a fibrin scaffold positioned over the site of infarction in swine hearts (55). Thus, a considerable amount of the benefit associated with engineered tissue transplantation may stem from the structural support of the graft or its cytokine production, in addition to direct remuscularization of the injured region.

Engineered Organs

Current limitations of human organ transplants have prompted researchers to consider use of xenogeneic organs as an alternative strategy. For example, functional pancreatata composed of rat cells have been generated in mice by injecting murine blastocysts with rat pluripotent stem cells (59), and then transplanting the blastocysts into surrogate mouse dams. Importantly, the blastocysts could not generate the target organ because they expressed a mutated form of Pdx1, the master regulatory gene for pancreatic development, and consequently provided a niche for the development of the wild-type rat organ. This “blastocyst complementation” strategy has also been used to produce livers and kidneys in rodents and pancreatata in pigs (60–62), whereas members of the Garry laboratory used an analogous approach that combined gene editing with somatic cell nuclear transfer to engineer pig embryos that lacked cardiovascular cells, and then rescued this deficiency with wild-type, green fluorescent protein–labeled pig blastomeres. Although these studies support the feasibility of generating patient-specific organs that can be used as models for preclinical work or, perhaps, as organs for transplantation therapy, the utility of this technology will

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remain limited until methods for generating organs from human stem cells become more efficient.

Stimulating Endogenous Regeneration and Repair

In addition to transplantation of exogenous stem cells and engineered tissues or organs, the ability to stimulate endogenous cardiac repair could eventually lead to development of effective cell-free therapies for MI. Lower organisms, such as the newt and zebrafish, as well as neonatal mice, have a tremendous ability to regenerate from severe myocardial injury (22), but the regenerative capacity of adult mouse and human hearts is much more limited. Nevertheless, studies that map cell fate or use radiocarbon dating indicate that both murine and human cardiomyocytes are continually replaced, albeit at a very low rate: an average of ~1% of human cardiomyocytes are newly formed each year, with roughly one-half of the cells replaced over a lifetime (59). A number of studies indicate that adult hearts can be remuscularized through the proliferation and differentiation of c-kit$^+$ CPCs, and endogenous CPCs may also release exosomes or paracrine factors that modulate the repair process and promote neovascularization. Nevertheless, genetic fate-mapping assessments by van Berlo et al. (63,64) indicated that although c-kit$^+$ CPCs can give rise to cardiomyocytes, they do so in an extremely limited fashion. Newly emerging studies are showing that endogenous repair mechanisms can be dramatically up-regulated (65).

Furthermore, although the fibroproliferative response (i.e., scar formation) is beneficial for short-term stability at the injury site, it interferes with subsequent repair processes, such as vascular growth and potentially remuscularization. Thus, researchers have also begun to investigate methods for controlling or reverting fibrosis by reprogramming fibroblasts into cardiomyocytes or ECs (66–68), and by identifying the cellular source(s) contributing to scar formation (69,70). The use of tissue-engineered systems may increase in vitro efficacy and improve understanding of the direct cardiac reprogramming processes (71,72), as well as permit well-controlled mechanistic studies of cardiomyocyte/nonmyocyte interactions (73).

Disparity between Preclinical and Clinical Study Results

The positive results from studies of cell therapy for the treatment of MI in small-animal models have generally not been observed in clinical trials. For example, results from a phase I clinical trial indicated that although intracoronary administration of autologous cardiac sphere-derived stem cells associated with a significantly decreased infarction size in patients with acute MI, LV chamber function did not improve (74). Many of the factors that determine the effectiveness of cell- or engineered-tissue–based therapies likely depend both on the unique characteristics of each specific disease state and on complex interactions among numerous mechanisms of action, but these variables cannot be adequately or safely explored in clinical investigations. Future tissue-engineering therapies for MI are expected to face the same logistic issues. Thus, relevant large-animal models of myocardial dysfunction are critical for identifying, characterizing, and quantifying the physiological response to cell and tissue transplantation therapies, as well as the optimal cell or biomaterial type, dose,

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timing, and route of administration, to ensure that each patient receives the maximum possible benefit while avoiding the complications associated with overtreatment.

Conclusions

Although adult bone marrow- or myocardium-derived progenitors can offer multiple paracrine benefits to surviving cardiomyocytes in the infarcted heart, they are unlikely to contribute to the formation of de novo working myocardium (75). Cardiomyocytes derived from pluripotent stem cells can address this challenge, but are more complicated to use in autologous therapies and potentially less robust to surviving transplantation. Although the jury for the optimal cell source is still out, it is possible that different disease applications will require different cell types, and that a mixture of immune-matched or immune-engineered PSC-derived cardiomyocytes and host-derived stromal progenitors will prove optimal in inducing heart remuscularization, while supporting cell survival and engraftment. Notably, applications of pre-formed engineered cardiac tissue patches with specifically tailored cell compositions could significantly increase both the survival and the beneficial effects of transplanted cells. Furthermore, because paracrine factors, including extracellular vesicles, are responsible for much of the observed beneficial effects of cardiac cell therapy, a maintained tissue patch could serve as a continued source of such beneficial paracrine signaling to the native heart tissue (Central Illustration). As our understanding of exosomal biology advances, patches can be engineered to optimize this signaling for cardiac regeneration. Use of genome-editing technologies may further enhance the potency and functional integration of delivered cells. Major challenges will be to address the potential arrhythmogenicity risks associated with a large graft. With exciting translational prospects ahead, future studies to optimize engineered cardiac tissue therapies in large animals and decipher the mechanisms of action are fully warranted.

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ABBREVIATIONS AND ACRONYMS

BMC	bone marrow–derived cell
CM	cardiomyocytes
CDC	cardiosphere-derived cell
EC	endothelial cell
IC	intracoronary
IM	intramyocardial
MHC	major histocompatibility complex

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MI	myocardial infarction
MSC	mesenchymal stem cell
PSC	pluripotent stem cell

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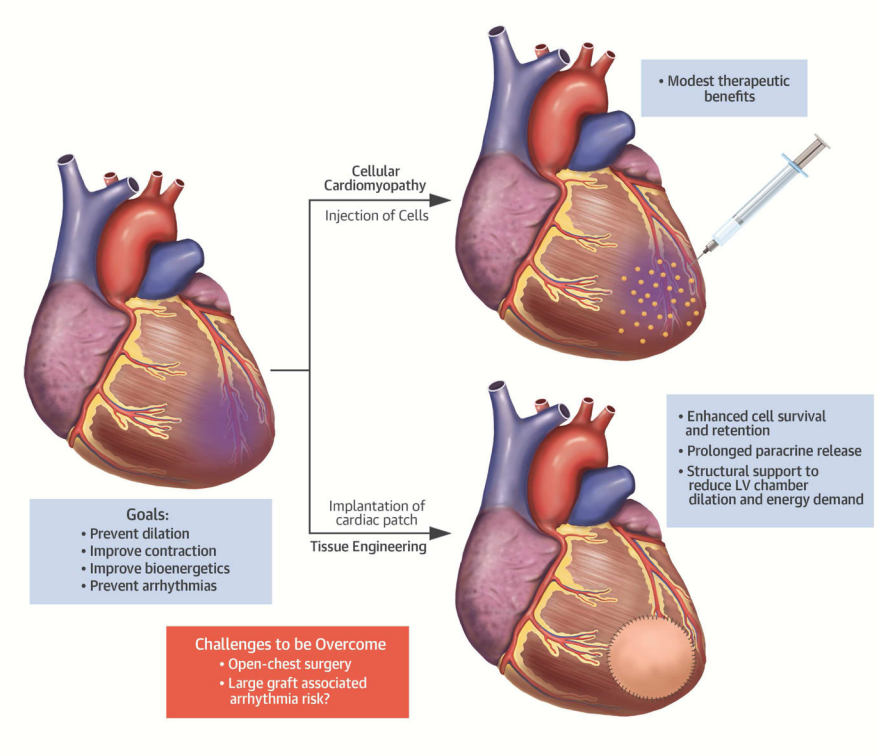

Central Illustration. Overview of Strategies to Overcome the Roadblocks in Cardiac Cell Therapy

The delivery of types of cells generally resulted in modest therapeutic benefits. Transplantation of prefabricated engineered heart tissue (e.g., cardiac tissue patch containing pluripotent stem cell–derived tri-cardiac cells) could enhance the therapeutic effects by an increased engraftment rate, which in turn, results in prolonged release of cytokines, reduction in left ventricular (LV) dilation and LV wall stresses. A major challenge that remains to be addressed is the potential arrhythmia risks associated with a large graft.

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Table 1

Published Stem/Progenitor Cell Clinical Trials for Heart Diseases

Diseases	Trial Design	Sample Size	Cell Type	Cell Source	Delivery Route	Follow-Up	Summary/Observation		Trial Name/Identifier
							Heart Function	Others	
HLHS	Nonrandomized (phase 1)	7	Autologous CDC	Cardiosphere	IC	18–36 months	↑ RVEF	Improved somatic growth	TICAP/NCT01273857 (76,77)
HLHS	Randomized (phase 2)	34	Autologous CDC	Cardiosphere	IC	12 months	↑ RVEF	Reduced fibrosis, improved somatic growth	PERSEUS/NCT01829750 (78)
Ischemic heart disease	Randomized control (phase 2)	90	Autologous BMCs	Bone marrow	IC vs. IM	6–12 months	↑ LVEF in IM group but not in IC group	↓ NT-proBNP in IM group but not IC group	REGENERATE-IHD/NCT00747708 (79)
Ischemic heart disease	Randomized control (phase 3)	271	Autologous MSCs	Bone marrow	IM	39 weeks	No change LVEF	↓ incidence of sudden or aborted sudden deaths	CHART-1/NCT01768702 (80)
Nonischemic cardiomyopathy	Randomized control (phase 2)	22	Allogeneic MSCs	Bone marrow	IV	90 days	No change LVEF	↑ health status and ↓ circulating inflammatory cells	NCT02467387 (81)
Refractory Angina	Randomized control (phase 2)	31	Autologous $CD133^+$ cells	Bone marrow	TEP	1, 4, 6, and 12 months	No change LVEF	↓ angina	REGENT-VSEL/NCT01660581 (82)
Ischemic vs. nonischemic dilated cardiomyopathy	Nonrandomized (phase 1)	27	Autologous skeletal stem-cell sheets	Skeletal muscle (vastus medialis)	Sutured to heart surface	12 months	↑ LVEF in patients with ischemic heart diseases, but not in patients with nonischemic heart diseases	↓ BNP in patients with ischemic heart diseases, but not in patients with nonischemic heart diseases	UMIN000003273 (83)
Nonischemic dilated cardiomyopathy	Randomized (phase 2)	37	Allogeneic MSC (19 patients) vs. autologous MSC (18 patients)	Bone marrow	TEP	12 months	↑ LVEF in allo-MSC group, but not in the auto-MSC group	↓ TNF-α, to a greater extent with allo-hMSCs versus auto-hMSCs at 6 months	POSEIDON-DCM/NCT01392625 (24)
STEMI	Randomized control (phase 2)	188	Autologous BMCs	Bone marrow	IC	6 months	↑ LVEF in BMC group	No treatment effect in irradiated BMC group	BOOST-2/ISRCTN17457407 (84)

BMC = bone marrow–derived cell; BNP = B-type natriuretic peptide; CDC = cardiosphere-derived cells; HLHS = hypoplastic left heart syndrome; hMSC = human mesenchymal stem cell; IC = intracoronary infusion; IM: = intramyocardial injection; IV = intravenous; LVEF = left ventricular ejection fraction; MSC = mesenchymal stem cell; NT-proBNP = N=-terminal pro-B-type natriuretic peptide; RVEF = right ventricular ejection fraction; STEMI = ST-segment elevation myocardial infarction; TEP = transendocardial injection; TNF = tumor necrosis factor.