Stem Cells and the Future of Heart Transplantation

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Abstract

Allogenic heart transplantation remains the only curative therapy for heart failure. Several strategies have been proposed including cell replacement therapy, engineered cardiac tissues, and novel transplant grafts derived from decellularized organs or xenotransplantation. Cell replacement therapy is the most mature of these technologies, but despite decades of clinical investigation, cardiac cell therapy has yet to enter cardiovascular practice. The major obstacle to replacing lost or injured myocardium remains a reproducible source of electro-, mechano-, and immunocompatible cardiomyocytes. Noncontractile cells like bone marrow or adult heart derivatives neither engraft long-term nor induce new muscle formation. Correspondingly, these cells offer little functional benefit to infarct patients. In contrast, transplantation of bona fide cardiomyocytes derived from pluripotent stem cells produces direct remuscularization. This new myocardium beats synchronously with the host heart and induces substantial contractile benefits. This chapter reviews the recent progress made

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toward novel cardiac transplantation strategies with attention to the underlying mechanisms of benefit to appreciate the barriers to cardiac repair and establish a rational path for optimizing therapeutic benefit.

Keywords

Stem cell · Cell therapy · Cardiac remuscularization · Transplantation

Introduction

Pioneered over half a century ago, allogenic heart transplantation remains the only curative therapy for heart failure. Substantial advances in patient selection, operative technique, and optimization of medical therapy and immunosuppression have improved outcomes and quality of life for patients eligible and fortunate to receive a compatible heart. However, supply of donor organs remains relatively unchanged despite rapidly increasing incidence of heart failure. As such, demand for organs continues to far outpace supply worldwide (Khush et al. 2018). The search for alternative sources of cells, tissues, and organs to restore and replace failing hearts is an active and increasingly diverse field of research.

Seminal advances in developmental biology now make novel methods for the transplantation of cell-, tissue-, and organ-based grafts clinically plausible although none are ready yet for clinical use. While evolution of this field will require a spectrum of tailored technologies to replace dysfunctional or deficient myocardium from diffuse disease processes, present efforts focus almost entirely on treatment of ischemic cardiomyopathy. Myocardial infarction (MI) is the most prevalent driver of heart failure and represents a demarcated loss of contractile myocardium supplanted by stabilizing scar. Use of stem cell-derived cardiomyocytes to remuscularize an infarcted territory is intuitive and supported by preclinical studies in small and large animal models. For other cardiomyopathies where disease affects the entire heart, large tissue grafting or organ transplantation may be required. The sources for such transplants as well as general considerations and challenges of each strategy will be reviewed in this chapter.

Cardiac Cell Therapies

Skeletal Myocytes: The first cell type transplanted to directly remuscularize infarcted myocardium was adult skeletal myoblasts over two decades ago (Murry et al. 1996; Taylor et al. 1997). While initially hypothesized to transdifferentiate into cardiomyocytes, this has been conclusively shown not to be the case (Reinecke et al. 2002), and the cells themselves do not electromechanically couple with the host myocardium (Dib et al. 2005; Reinecke et al. 2000). Early clinical trials showed promising benefit, but effects proved transient and appear mediated through noncontractile, paracrine mechanisms (Menasche et al. 2008; Povsic et al. 2011; Taylor et al. 1998). Pivotal clinical trials of autologous skeletal myoblast transplantation in patients with heart failure did not durably improve regional or global left ventricular (LV) function and caused persistent ventricular arrhythmias (Fouts et al. 2006), prompting abandonment of this cell type for therapy (Menasche et al. 2008).

Non-myocyte Stem Cells: More recent efforts have shifted to other adult sources of cells purporting regenerative benefit through cell-cell and paracrine mechanisms, activating and stimulating endogenous regeneration, and modulating repair processes. Numerous autologous and -allogeneic adult cell types have been investigated clinically including adult-derived cells of cardiac origin such as cardiosphere-derived cells (CDCs) and non-cardiac origin such as various bone marrow (BM)-derived cells [e.g., BM-derived mononuclear stem cells (BM-MNCs) and mesenchymal stromal cells (BM-MSCs)] (Cambria et al. 2017; Menasche 2018). These so-called "first-generation" cell types have been further refined as "second-generation" cells composed of purified or cytokine-stimulated subpopulations to potentiate their regenerative capacity. In all, 15 types of adult-derived cells have shown benefit in small animal models of myocardial infarction (Cambria et al. 2017).

Investigators have hypothesized multiple effects of these cells in addition to regeneration including paracrine secretion of cardioactive cytokines and growth factors, leading to expanded indications for cell therapy from acute myocardial infarction, where preclinical evidence was already lacking, to ischemic and nonischemic cardiomyopathy to refractory angina (Perin et al. 2012), peripheral artery disease (Losordo et al. 2012), and stroke (Misra et al. 2012). Notably, the field of adult cell transplantation is marred by the recent retraction of multiple papers from a single lab on the regenerative capacity of BM-derived adult "stem" cells and, secondly, the existence of an endogenous cardiac stem cell phenotype as c-kit^{+} (Chien et al. 2019). Nevertheless, in preclinical models, diverse cell therapies may have modest benefit through noncontractile mechanisms. These effects do not appear restricted to adult-derived cells, and their derivatives give the remarkable heterogeneity of cells that appear efficacious. For example, pluripotent-derived cardiomyocytes ectopically transplanted in engineered scaffolds have shown similar benefit as adult-derived cells in infarcted pigs, despite such grafts failing to couple electromechanically or vascularize with the host myocardium (Gao et al. 2017; Gerbin et al. 2015; Jackman et al. 2018; Shadrin et al. 2017; Weinberger et al. 2016). In one of the most rigorous mechanistic studies specifically of adult cell therapy, a preliminary report from Vagnozzi, Molkentin et al. describe a novel innate immune response that explains the benefit through induction of a specific subset of macrophages to modulate wound healing in the infarct area (Vagnozzi et al. 2018). Taken collectively, the poorly understood yet reproducible benefit of noncontractile cell transplantation in various disease states appears remarkably conserved across various cardiac and non-cardiac cells suggesting a non-specific effect of cell therapy of modest clinical benefit without concomitant restoration of contractile myocardium.

Therapeutic development of these adult cell types has been accelerated to numerous phase 2/3 clinical trials within the past decade prior to clear mechanistic understanding of their function (Broughton and Sussman 2016; Madonna et al. 2016). Trials to date have generally employed heterogeneous populations of adult cell types and have, for the most part, shown safety regardless of the specific investigational cell product, delivery approach, dosing protocol, or patient characteristics. Individual trials initially suggested efficacy, but these early trials were small without randomization, standardized enrollment criteria, endpoints, or adjudication. More recent trials with larger cohorts and superior study design have generally failed to convincingly show benefit over guideline-directed medical therapy (Fernández-Avilés et al. 2017; Fisher et al. 2015; Gyongyosi et al. 2016; Madonna et al. 2016) (Table 1). A recent Cochrane meta-analysis of 38 randomized controlled trials capturing 1,907 post-MI patients concluded the that the current body of evidence for cell therapy to be low quality and lacking evidence for benefit by composite endpoint of mortality, nonfatal myocardial infarction, and/or heart failure readmission (Fisher et al. 2016). Long-term mortality >12 months and incidence of nonfatal myocardial infarction were individually reduced with cell therapy, but confounded by relatively low event rates, small study cohorts, and non-standardized trial designs and adjudication.

Pluripotent Stem Cells: Human pluripotent stem cells, a renewable source of all somatic cell types including cardiomyocytes, have received the most study for application in regenerative therapies. The availability of well-characterized cardiomyocytes in substantial and reproducible quantities enables novel cell-, tissue-, and organ-based therapies. Both human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have been used as a renewable source of differentiated cardiomyocytes.

First isolated in 1998 (Thomson et al. 1998), human ESCs have been characterized for decades and specific lines with favorable attributes for clinical development such as karyotypic stability, facile differentiation into the cell of interest, and ethical sourcing. ESCs are isolated from the inner cell mass of the blastocyst in the early stages of embryogenesis and retain the potential to

Table 1 Select randomized controlled trials of adult cell transplantation for myocardial infarction and ischemic cardiomyopathy

Study	Design	Number of subjects		Cell type	Route	Cell number ($\times 10^6$)	Timing (post-AMI)	Follow-up (mo)	Primary outcome	Result
		T	C							
Acute myocardial infarction										
BOOST (Wollert et al. 2004)	SC, OL	30	30	Allo BM-MNC	IC	2460	5–7 days	6	Global LVEF	Positive
REPAIR–AMI (Schachinger et al. 2006)	MC, DB	95	92	Auto BM-MNC	IC	236 ± 174	3–6 days	4	Global LVEF	Positive
Leuven-AMI (Janssens et al. 2006)	SC, DB	33	34	Auto BM-MNC	IC	172 ± 72	24 h	4	Global LVEF	Negative
FINCELL (Huikuri et al. 2008)	MC, DB	39	38	Auto BM-MNC	IC	402 ± 196	3 days	6	Global LVEF	Positive
REGENT (Tendera et al. 2009)	SC, OL	97	20	Auto BM-MNC or $CD34^+CXCR4^+$ BM-MNC	IC	178 (BM-MNC) 1.90 ($CD34^+CXCR4^+$ BMCs)	3–12 days	6	Global LVEF	Negative
BONAMI (Roncalli et al. 2011)	MC, OL	52	49	Auto BM-MNC	IC	98.3 ± 8.7	9 days	3	Myocardial viability	Negative
HEBE (Hirsch et al. 2011)	MC, OL	69	65	BM-MNC/PB-MNC	IC	296 ± 164 (BM) 287 ± 137 (PB)	5–7 days	4	Global or regional LVEF	Negative
LateTIME Trial (Traverse et al. 2011)	MC, DB	58	29	Auto BM-MNC	IC	150	2–3 weeks	6	Global LVEF	Negative
REGENERATE-AMI (Choudry et al. 2016)	MC, DB	55	45	Auto BM-MNC	IC	60	<24 h	12	Global LVEF	Negative
SWISS-AMI (Sürder et al. 2016)	MC, DB	95	55	Auto BM-MNC	IC	152	5–7 days or 3–4 weeks	12	Global LVEF	Negative
PreSERVE-AMI (Quyyumi et al. 2017)	MC, DB	78	83	Auto CD34+ cells	IC	10 ± 2	9 days	6	Resting myocardial perfusion	Negative

BOOST-2 (Wollert et al. 2017)	SC, OL	151	37	Allo BM-MNC	IC	700–2080	8.1 ± 2.6 days	6	Global LVEF	Negative
TIME (Traverse et al. 2012, 2018)	MC, DB	79	41	Auto BM-MNC	IC	147 ± 17	3 or 7 days	6 and 24	Global or regional LVEF	Negative
CAREMI (Fernández-Avilés et al. 2018)	MC, DB	33	16	Allo BM-c-kit$^+$ CSC	IC	35	5–7 days	1	Safety, all-cause mortality, reinfarction, HF hospitalization, VT/VF, stroke)	Negative
BAMI, NCT01569178 (Mathur et al. 2017)	MC, DB	~175	~175	Auto BM-MNC	IC	n/a	2–8 days	24	All-cause mortality	Recruiting, est. completion 2019
Ischemic cardiomyopathy										
MAGIC (Menasche et al. 2008)	MC, DB	67	30	Skeletal myoblasts	TEP	400 or 800	>1 mo		Global or regional LVEF	Negative
FOCUS-HF (Perin et al. 2011)	SC, OL	20	10	Auto BM-MNC	TEN	178	>3 mos	12	QOL, MLHFQ	Positive
FOCUS-CCTRN (Perin et al. 2012)	MC, DB	61	31	Auto BM-MNC	TEN	100	>1 mo	6	LVESV, VO_2 max, SPECT reversibility	Negative
POSEIDON (Hare et al. 2012)	SC	31	0	Allo or auto BM-MSC	TEN	20, 100, or 200	n/a	1	Treatment-emergent serious adverse events	n/a
CELLWAVE (Assmus et al. 2013)	SC, DB	82	40	Auto BM-MNC	IC	205 ± 110	>3 mos	4	Global LVEF	Positive
TAC-HF (Heldman et al. 2014)	SC, DB, sham control	38	21	Auto BM-MNC or auto BM-MSC	TEN	100 or 200	n/a	12	Treatment-emergent serious adverse events	Neutral
MSC-HF (Mathiasen et al. 2015)	SC, DB	40	20	Auto BM-MSC	TEN	77.5 ± 67.9	>6 weeks	6	LVESV	Positive
ixCell-DCM (Patel et al. 2016)	MC, DB, sham control	58	51	Proprietary auto BM-MSC and M2 macrophages	TEN	n/a	>3 mos	12	Composite (all-cause death, cardiovascular hospitalizations, worsening HF, etc.)	Positive

(continued)

Table 1 (continued)

Study	Design	Number of subjects		Cell type	Route	Cell number ($\times 10^6$)	Timing (post-AMI)	Follow-up (mo)	Primary outcome	Result
		T	C							
CHART-1 (Bartunek et al. 2017)	MC, DB, sham control	120	151	Auto BM-MSC (CpSC)	TEN	24	>3 mos	40	Composite (all-cause death, worsening HF, MLHFQ, 6MWT, LVESV, and LVEF)	Negative
REGENERATE-IHD (Choudhury et al. 2017)	SC, DB	70	35	G-CSF/auto BM-MNC	IC or TEN	115.1	>3 mos	12	Global LVEF	Positive for TEN
POSEIDON-DCM (Hare et al. 2017)	SC	37	0	Allo or auto BM-MSC	TEN	100	n/a	12	Treatment-emergent serious adverse events	Neutral
CONCERT-HF (NCT02501811) (Bolli et al. 2018)	MC, DB	~72	~72	Auto BM-MSC + c-kit$^+$ CSC	TEN	n/a	n/a	12	Global LVEF, VO_2 max, 6MWT, etc.	Paused
DREAM HF-1 (NCT02032004)	MC, DB	~300	~300	Auto BM-MSC (MPCs)	TEN	n/a	n/a	12	Time to HF exacerbation	Paused
CHART-2 (NCT02317458)	MC, DB, sham control	~200	~200	Auto BM-MSC (CpSC)	TEN	n/a	n/a	52	Composite (CV death, worsening HF, MLHFQ)	Canceled
CardiAMP (NCT02438306) (Raval et al. 2018)	MC, DB, sham control	167	83	Potency-screened auto BM-MNC	TEN	200	n/a	12	6MWT	Recruiting, est. completion 2021
REPEAT (NCT01693042) (Assmus et al. 2016)	MC, OL	~334	~334	Auto BM-MNC	Repeated IC	n/a	n/a	24	All-cause mortality	Recruiting, est. completion 2025

differentiate into any somatic cell type given the appropriate stimulation. Initially, there was hope that the heart milieu itself could provide either critical cell-cell cues or growth factors to guide ESCs to a cardiac phenotype and integrate into host myocardium. This notion was quickly dispelled as injected ESCs into mouse myocardium formed teratomas rather than mature cardiomyocytes (Nussbaum et al. 2007) in addition to eliciting immunogenicity and graft rejection (Swijnenburg et al. 2005). Cardiomyocytes derived from human ESCs, however, can be transplanted and survive in normal rodent hearts (Laflamme et al. 2005) and electrically couple with existing cardiomyocytes in porcine models (Kehat et al. 2004). When transplanted into recipient rodent models after MI, there was a reproducible and durable improvement in LV function and electrical coupling with the host myocardium (Caspi et al. 2007; Laflamme et al. 2007; Mummery et al. 2003; Qiao et al. 2011; Shiba et al. 2012).

The discovery of an alternative pluripotent stem cell, induced pluripotent stem cells (iPSCs), by Takahashi and Yamanaka et al. in 2007 has markedly accelerated pluripotent stem cell research and translation into potential therapies (Takahashi et al. 2007). Overexpression of four genes (c-Myc, Oct3/4, SOX2, and Klf4) known to maintain pluripotency in stem cells reprogrammed somatic cells back to a state of pluripotency. The process has been validated using a full spectrum of somatic cells including cells isolated from a single hair follicle or a sample of blood. iPSCs offer benefits over ESCs such as autologous source, allowing patient-specific HLA compatibility, potentially obviating the need for immunosuppression, and avoiding the societal issues surrounding blastocyst and embryo research. However, the reprograming process has been reported to result in genomic abnormalities and incomplete reprogramming, leaving residual epigenetic marks that are of uncertain clinical significance (Kim et al. 2010). Cardiomyocytes generated from iPSCs, however, appear functionally indistinguishable from cardiomyocytes derived from ESCs and native cardiomyocytes, albeit with similar immaturity as all pluripotent-derived myocytes (Schenke-Layland et al. 2008; Zhang et al. 2009).

Preclinical proof-of-concept studies of pluripotent stem cell-derived cardiomyocytes are increasingly promising as the field transitions from in vitro and small animal models to more relevant large animal studies (Milani-Nejad and Janssen 2014; van der Spoel et al. 2011). Efficient methods for high-purity, clinical-grade cardiomyocyte production from ESCs now allow extension of transplant cell strategies into preclinical large animal studies (Thies and Murry 2015). Murry and colleagues have transplanted one billion human ESC-derived human cardiomyocytes (hESC-CMs), approximating the cell loss during human myocardial infarction, to successfully create a large tissue graft in subacutely infarcted nonhuman primates (Chong et al. 2014). In this study, hESC-CMs were surgically injected into the hearts of immunosuppressed primates 2 weeks after infarction, resulting in significant remuscularization of the infarcted myocardium. The human graft became vascularized and electromechanically coupled with the host myocardium within 2 weeks posttransplant, and such grafts have remained durable up to 3 months (Fig. 1).

More recent examples demonstrate the effectiveness and durability of human pluripotent stem cell (hPSC)-CM transplantation up 3 months. Shiba et al. transplanted 400 million nonhuman primate (NHP)-induced pluripotent stem cell (iPSC)-derived cardiomyocytes into MHC-matched NHPs with follow-up to 3 months (Shiba et al. 2016). Following transplantation, global contractility improved at 1 month and was sustained at 3 months compared to cell-free vehicle treatment. Importantly, this allogenic transplantation study expands our understanding of the immunology of hPSC-CM grafts. With MHC homozygosity, grafts were supported without rejection up to 3 months using a moderate immunosuppression regimen commonly used clinically. The minimum immunosuppression required for MHC-matched, PSC-CM allografts was not tested, but this study suggests that immunotolerance of these grafts is possible

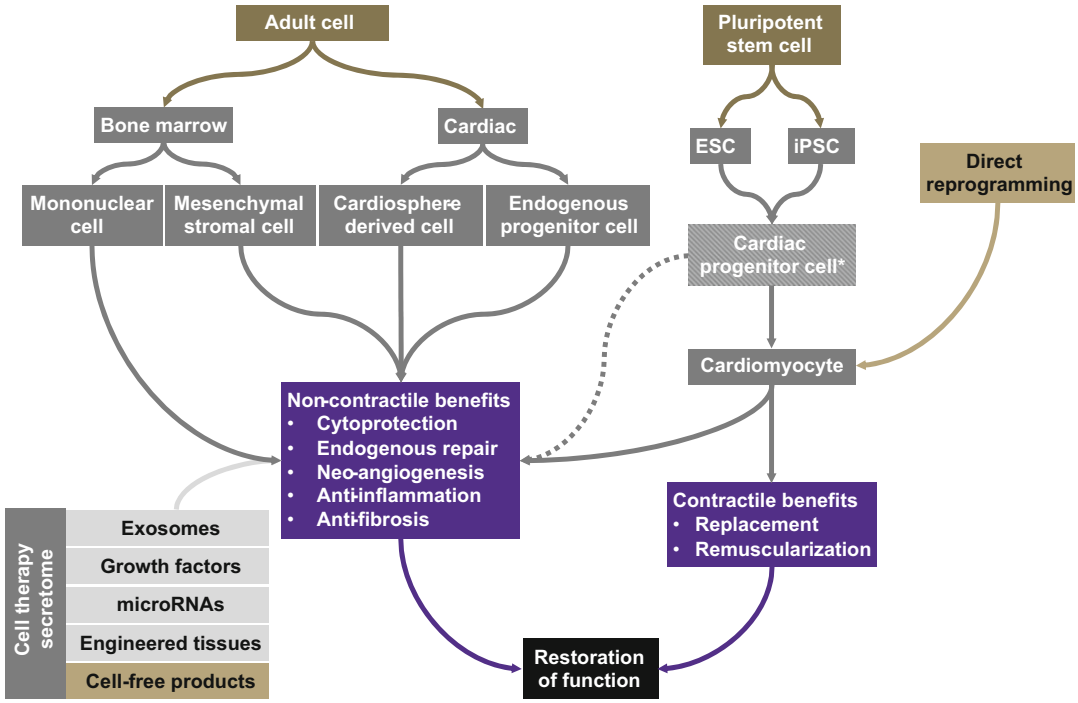

Fig. 1 Function follows form: proposed cell types for therapeutic cardiac regeneration. (*Cardiac progenitor cell is theoretical given populations such as c-kit^{+} cell have been proven not to be cardiopoietic)

without the prohibitive immunosuppression required for xenotransplantation.

Murry et al. recently reported the long-term functional benefit of 750 million hESC-CM in nonhuman primates (Liu et al. 2018). Improved contractile function was again seen at 1 month, and at 3 months, function continued to improve to fully normalize left ventricular function with hESC-CM therapy (Fig. 2). Control subjects negatively remodeled during the study with a continual decline in LV function over time as expected without background medical therapy. The persistent and cumulative benefit of engrafted hESC-CM both subacutely and chronically may reflect the importance of cellular engraftment to exert continuous benefit through both contractile and noncontractile mechanisms. Dissecting the relative contribution of each in this setting is challenging. Whereas prior attempts at cardiac regeneration did not result in meaningful retainment of cell product, and thus any benefit can be safely attributed to noncontractile benefit, hPSC-CM transplantation clearly results in durable engraftment. While observation of large-scale remuscularization with contractile and electromechanically coupled grafts suggests a direct functional benefit, conclusive evidence requires careful genetic and pharmacologic studies to isolate contractile from noncontractile effects. Mechanistic studies to investigate the relative contribution of contractile and noncontractile effects will be important to understand the core mechanisms of benefit to maximize efficacy of this promising technology while minimizing complications such as malignant tachyarrhythmias.

A speculative model may be that the hPSC-CM transplantation uniquely matches the natural history of an evolving MI with both noncontractile and contractile effects. Early post-injury, hPSC-CM may impart immediate and critical benefit to the subacute infarct by stimulating paracrine-mediated repair and moderation of injury. Indeed, pilot small animal studies have failed to show benefit of remuscularization in chronic ischemic cardiomyopathy (Fernandes et al. 2010; Shiba

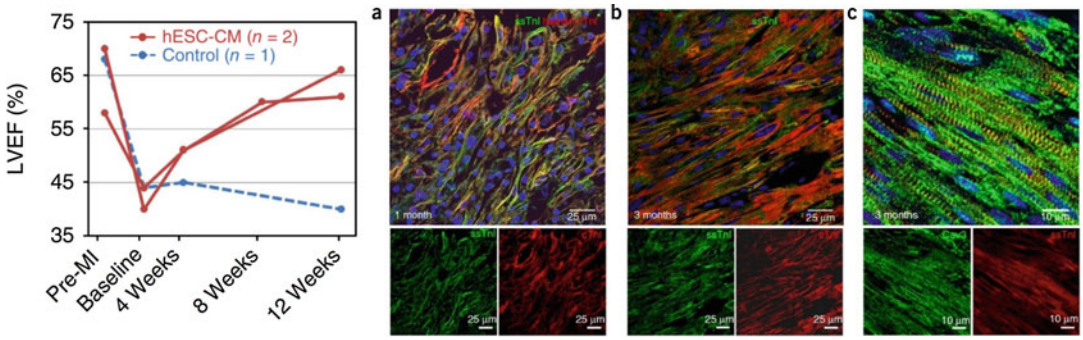

Fig. 2 Long-term benefit maturation of hESC-CM therapy following subacute myocardial infarction in nonhuman primate. Left panel: Benefit of hESC-CM therapy continues up to 3 months (red) compared continued functional decline in control-treated subject (blue). Right panel: hESC-CM grafts stained for slow skeletal troponin I (ssTnI, green) and human cardiac troponin I (cTnI, red). Merged image on top, individual channels below. Scale bar, 25 μm. (**a**) At 1 month the hESC-CMs are relatively small, have peripheral myofibrils, and exhibit low cellular alignment. Low-level expression of cTnI is evident. This experiment was repeated in two biologically independent hESC-CM-treated hearts with similar results. (**b**) At 3 months the cells have hypertrophied, have myofibrils throughout the cytoplasm, and are better aligned. Increased expression of cTnI is evident. This experiment was repeated in two biologically independent hESC-CM-treated hearts with similar results. (**c**) At 3 months, graft T-tubule networks are present, shown by caveolin-3 staining (Cav3, green). ssTnI, red. This experiment was repeated in two biologically independent hESC-CM-treated hearts with similar results. Scale bar, 10 μm. Abbreviations: cTnI, cardiac troponin, isotype I; hESCM-CM, human ESC-derived human cardiomyocytes; LVEF, left ventricular ejection fraction; MI, myocardial infarction; ssTni, slow skeletal troponin, isotype I. (Reproduced from Liu et al. 2018)

et al. 2014), suggesting a finite window of intervention for hPSC-CM remuscularization therapy to alter long-term disease trajectory. As cardiomyocytes are replaced with scar and the LV negatively remodels, the nascent cardiomyocyte graft is maturing and increasingly exerts contractile benefits including force generation and structural support. This transition parallels structural and electrical changes that occur in implanted hPSC-CM over the next 3 months resulting in higher sarcomeric organization and electrical quiescence. Indeed, hPSC-CM cells are fetal-like at the time of delivery which is a requisite phenotype to survive the hostile post-infarct myocardium and effectively engraft (Gerbin and Murry 2015; Zhang et al. 2009). The cells rapidly mature in vivo and ultimately contribute directly to contractile function and positive remodeling.

Despite the promise of hPSC-CM transplantation, significant challenges to clinical translation remain, including scaling cell manufacturing to clinical levels, graft tolerance and immunosuppression, tumorigenicity, delivery, and, most acutely, arrhythmogenesis (Stevens and Murry 2018; Thies and Murry 2015). In earlier work with mice, rats, and guinea pigs, no arrhythmias were observed after hESC-CM transplantation. When studies transitioned to macaques, however, a significant burden of ventricular arrhythmias is observed. Electrophysiological studies indicate that these arrhythmias result from ectopic pacemaking activity by the graft cells, rather than reentry due to heterogeneous tissue. These arrhythmias typically last for 2–3 weeks following implantation, after which the hearts return to normal sinus rhythm. The lack of arrhythmias in smaller animals likely relates to host heart rate. Heart rates in model species range from 600 (mouse) to 400 (rat) to 250 (guinea pig) beats per minute. Not until therapy was tested in nonhuman primate with a resting heart rate of 120–150 bpm were ventricular arrhythmias reproducibly observed. One current hypothesis is that arrhythmias stop when there is enough electrical maturation to drop pacemaker rates by the graft below that of the sinus node. Although the ventricular arrhythmias are tolerated by

macaques, it is likely that they will pose an increasing challenge in larger hearts like those in pigs and humans.

Other barriers to hESC-CM therapy include efficient and reproducible cell production and processing, graft survival without prohibitive immunosuppression, and, at present, surgical epicardial delivery, all of which must be addressed prior to clinical feasibility. To circumvent many of these issues, an alternative strategy employing a surgically placed epicardial patch seeded with ESC-derived cardiac progenitor cells is already enrolling a first-in-human trial (Menasche et al. 2015) despite recent evidence suggesting that cardiac progenitors do not durably engraft and any benefit is mediated through transient paracrine mechanisms (Zhu et al. 2018).

Engineered Cardiac Tissues

For over two decades, cardiomyocytes have been cultured in functional three-dimensional matrices to mimic the structure of cardiac tissue. Transplantation of engineered cardiac tissues, rather than an inoculum of cells, offers several potential advantages: (1) more efficient graft retention, (2) better structural support, (3) less arrhythmogenicity, and (4) immunoreactivity more similar to conventional organ transplants. The first functional engineered cardiac tissue was reported by Eschenhagen et al. in 1997 with chick embryonic cardiomyocytes seeded onto a collagen hydrogel matrix that was successfully paced and maintained force generation for 11 days (Eschenhagen et al. 1997). Use of mammalian cells into increasing complex matrices with structures resembling or derived from actual native human myocardium has permitted the use of engineered cardiac tissues in myriad in vitro and in vivo applications. Intuitively, cardiomyocyte maturity, a significant limitation to current cardiomyocyte differentiation protocols, is enhanced when cultured in three-dimensional matrices compared to traditional two-dimensional monolayer cultures (Nunes et al. 2013; Ronaldson-Bouchard et al. 2018; Zhang et al. 2013).

A logical strategy is to use human pluripotent stem cells as an abundant source of cardiomyocytes added to an engineered matrix to create phenotypically acceptable cardiac tissue (Oikonomopoulos et al. 2018). Further advances have scaled these tissue constructs to physiologically relevant dimensions of 10–20 cm^2. These constructs have adequate force generation for therapeutic use and engraft and vascularize with host myocardium (Shadrin et al. 2017). Combined with advanced nano- and micro-fabrication techniques, cardiomyocytes of uniform alignment can be seeded to improve tissue anisotropy. Engineered cardiac tissue transplantation has reported encouraging improvements in function follow myocardial infarction models in rodent (Jackman et al. 2018; Riegler et al. 2015; Shadrin et al. 2017; Weinberger et al. 2016; Wendel et al. 2015) and pig (Gao et al. 2018; Kawamura et al. 2017). Challenges to clinical translation include diffusion limitations to graft thickness, long-term survival of cardiomyocytes, and most significantly lack of electromechanical integration with the host (due to formation of a non-cardiac barrier layer, intervening epicardial fat or dense infarct scar) (Gao et al. 2017; Gerbin et al. 2015; Jackman et al. 2018; Shadrin et al. 2017; Weinberger et al. 2016). The limitation of this technology to directly improve function was demonstrated by hESC-CMs seeded onto an engineered cardiac tissue transplanted into infarcted athymic rats did not show difference in LV ejection fraction up to 1 month whether viable or lethally irradiated cells were used (Riegler et al. 2015). Another limitation with these studies is the fact that the engineered tissues are transplanted minutes following open chest infarction, a timeframe where noncontractile paracrine effects are expected to be maximally beneficial through modulation of the post-infarct, inflamed, and hostile milieu while augmenting repair mechanisms. Translating such studies clinically, however efficacious, would be challenging given the current medical- and percutaneous-based standard of care for acute myocardial infarction. Recently, Menasche et al. (Menasche et al. 2015, 2018) transplanted a 20 cm^2 fibrin scaffold seeded with hESC-derived cardiac progenitor cells into six

patients with severe ischemic cardiomyopathy at the time of coronary artery bypass surgery. Concomitant revascularization of the treatment territory as well as lack of a control cohort severely limits interpretation of the pilot study. The therapy appeared safe without tumorigenicity or arrhythmogenesis during 6 months of follow-up as would be expected given the limited survival and integration at present with engineered cardiac tissues.

A variation of the technology is scaffold-free cardiac tissues developed by Okano et al. (Kawamura et al. 2017). Using a thermosensitive culturing surface, monolayers of up to 100 million iPSC-derived cardiomyocytes can be detached as intact sheets that can be stacked to create a three-dimensional tissue construct up to 0.1 mm thick and 10–20 cm^2 free of a foreign extracellular matrix. These cell-sheet tissues adhere to host epicardium without the need for anchors or suture thus further limiting immunoreactivity and coverage of the graft with omentum during surgical delivery appears to enhance perfusion and graft survival up to 3 months. The technology has shown benefit in various preclinical animal models of ischemic cardiomyopathy (Kawamura et al. 2017; Shimizu et al. 2009), and a clinical trial has been approved in Japan. Without synchronous contractility with the host and long-term cell survival, these patches are unlikely to contribute directly to host mechanical function and are essentially a vehicle to delivery modest paracrine, noncontractile benefits albeit over potentially long durations.

Single Ventricular Transplantation

Efforts to create entire ventricles for transplantation leverages similar technology as engineered cardiac tissues, endeavoring to instead form an entire chamber. An early attempt a "pouch-like" single ventricle construct, created by culturing neonatal rat cardiomyocytes in a casting mold, that was fitted over infarcted rat hearts (Yildirim et al. 2007). Presented as a biologic ventricular support prosthetic, the transplant does not integrate with the host or impart any contractile benefit. Other groups have reported alternative methods using hPSC-CM with generation of contractile forces as demonstrated in pressure-volume loops (Li et al. 2018) and within bioreactors (MacQueen et al. 2018). This strategy faces the same obstacles as cardiac tissues but further challenged by present-day limitations of cardiomyocyte production and anticipated difficulties of scale and delivery for clinical use.

Whole Heart De-/Recellularization

The complexity of the human heart is built upon the highly specialized architecture of an extracellular matrix (ECM) composed of structural and membrane proteins together with bioactive glycosylated protein groups (Rienks et al. 2014). The ECM orchestrates the development and function of 10 billion cells at every plane of biology. At the molecular and cell levels, paracrine signaling and adhesion mediate cardiac development and homeostasis, and at the tissue and organ, biophysical properties such as rigidity and elasticity and conductance of electrical and mechanical forces provide proper form and function. The critical role for ECM is the premise for whole heart de-/recellularization. Various synthetic ECMs have been proposed and have been reviewed recently (Pomeroy et al. 2019). Readily available and appropriate in size, the pig heart is an ideal source for ECM. Decellularization techniques strip >98% of porcine material (Guyette et al. 2014; Hodgson et al. 2018), and several groups have attempted recellularization of porcine ECM which highlight the challenges of this strategy (Kitahara et al. 2016; Lu et al. 2013; Ott et al. 2008). Preservation of the decellularized ECM's functional bioactivity is critical to cellular repopulation of the tissue; however, removal of immunoreactive signatures is necessary to prevent xenograft rejection. To this end, decellularization of human heart tissues has been reported (Guyette et al. 2016; Sanchez et al. 2015), but with limited function and restricted by availability of human donors. Repopulation of the ECM with human cardiomyocytes is

presently primitive, and techniques are required to recellularize to restore organ biomechanics, electrical stability, and vascularization. Additionally, availability of the sheer number of cells required is presently technically limiting.

Xenotransplantation

Genetically modified pig hearts may be a clinically feasible alternative to resource-limited allografts (Mohiuddin et al. 2015). Strategies for immunotolerance are central to the field of xenotransplantation. However, orthotopic transplantation of pig heart into baboon has largely been unsuccessful despite over two decades of investigation. However, advances in attenuating the immunogenicity of pig hearts have allowed the generation of pig hearts that lack galactose-α1,3-galactose epitopes and express the human membrane marker CD46 and human thrombomodulin. These engineered hearts have survived for upward of two and half years of heterotopic xeno-implantation into the abdomen of a baboon host (Mohiuddin et al. 2016). Recently, orthotopic transplantation of a similar pig heart in combination with clinically viable immunosuppression successfully sustained the host for 195 days (Langin et al. 2018), a dramatic improvement from prior studies and if reproducible may herald clinical investigation.

Conclusion

The severe and endemic shortage of donor hearts continues to compel investigators in search of alternative sources and techniques for cardiac transplantation. There have now been over 100 clinical trials of cell therapy for acute myocardial infarction, over 90 for chronic ischemic cardiomyopathy, and 25 for nonischemic cardiomyopathy (Fernández-Avilés et al. 2017). The evolving technologies range from cell-based to whole organ strategies, each demonstrating early feasibility in preclinical studies or pilot clinical trials but also fraught with challenges often specific to the technology. Adult stem cell therapies have been by far the most extensively studied to date, and the disappointing clinical experience reveals the importance of fundamental mechanistic insight and provides a cautionary lesson for investigators considering first-in-human trials for novel transplant technologies to establish protocols through careful preclinical investigation and validation. Numerous open questions remain for the clinical translation of cardiac cell therapy (Madonna et al. 2016) (Fig. 3). Two decades of investigations in adult cell therapy provides a reassuring framework of clinical trial design and infrastructure for the safe delivery of cells in such trials. Paracrine-based strategies, however, likely will provide only modest benefit of unclear durability. Once better understood, these pathways may be an important adjunctive benefit for therapies based on contractile cell, tissue, or organ transplantation. To complement and eventually supersede orthotopic heart transplantation, therapies will require contractile-based mechanisms of action to functionally replace lost or dysfunctional myocardium. Nascent are technologies that inherently do not integrate electrically or mechanically with host myocardium. And while the lack of arrhythmia and immunogenicity is often cited as evidence of safety for such therapies, they are not unexpected through the lens of fundamental mechanism and may represent significant limitations to efficacy. Thus at present, the most promising preclinical investigations of cardiac remuscularization therapy return to the premise that meaningful and durable recovery of injured myocardium requires genuine and direct regeneration of lost myocardium to restore contractility (Bertero and Murry 2018; Eschenhagen et al. 2017; Thies and Murry 2015). The recognized challenges related to arrhythmogenesis, immunosuppression, and efficient cell production with direct cardiac cell replacement require solutions before clinical viability but may be intrinsic to the fundamental strategy of true cardiac remuscularization.

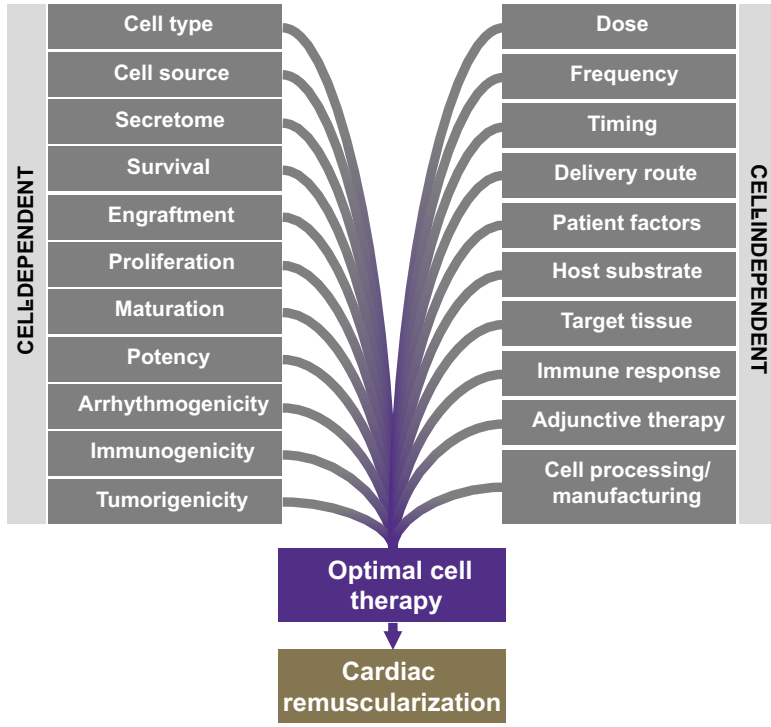

Fig. 3 Open questions in cardiac cell transplantation

Cross-References

- ▶ Pathophysiology of Heart Failure
- ▶ Will We Still Be Doing Heart Transplants in 10 Years?

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