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REGENERATIVE MEDICINE (SM WU, SECTION EDITOR)

Pluripotent Stem Cell-Derived Cardiomyocyte Transplantation for Heart Disease Treatment

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Abstract

Purpose of Review Cardiovascular disease is the leading cause of mortality worldwide. Pluripotent stem cell-derived cardiomyocytes (PSC-CMs) have great potential to treat heart disease, owing to their capacity of engraftment and remuscularization in the host heart after transplantation. In the current review, we provide an overview of PSC-CMs for clinical transplantation.

Recent Findings Studies have shown that PSC-CMs can survive, engraft, and form gap junctions after transplantation, with functional benefit. Engrafted PSC-CMs matured gradually in host hearts. Only in a large animal model, transient ventricular arrhythmias were detected, mainly because of the ectopic pacing from the grafted PSC-CMs. Although intense immunosuppression is unavoidable in xenotransplantation, immunosuppression remains necessary for MHC-matched allogenic non-human primate PSC-CMs transplantation.

Summary This review offers insights on how PSC-CMs contribute to functional benefit after transplantation to injured non-human primate hearts. We believe that PSC-CM transplantation represents a potentially novel treatment for ischemic heart diseases, provided that several technological and biological limitations can be overcome.

Keywords Cardiomyocyte · Pluripotent stem cell · Heart disease · Transplantation · Cell therapy

Introduction

Cardiovascular disease, which can lead to heart failure, is the leading cause of mortality and morbidity in the world [1]. Conventional therapy with pharmacological or surgical treatments can delay the progression of heart failure but cannot cure the disease. Heart transplantation is the only durable treatment to cure advanced heart failure at present; however, there is an insufficient supply of donor hearts worldwide. Over the past two decades, substantial studies have been carried out to regenerate the heart, such as cell therapy to replace injured cardiomyocytes. Following preclinical studies with animal models, clinical transplantation studies were performed with adult stem cells, such as bone marrow-derived cells [2–4], skeletal myoblasts [5], and resident cardiac stem cells [6, 7]. However, stem cell therapies using these cells have failed to become standard therapy for heart failure. This is mainly because transplanted adult stem cells can neither survive long-term nor differentiate into cardiomyocytes in the host tissue [8]. Usually, in the failing hearts from patients with ischemic heart disease, a substantial number of cardiomyocytes are lost and replaced by non-contractile fibroblasts. To regenerate the failing heart, a large number of cardiomyocytes are needed that survive long term and replace dead cardiomyocytes.

Pluripotent stem cells (PSCs: ESCs and iPSCs, embryonic and induced pluripotent stem cells) are promising cell sources because of their unlimited self-renewing ability and unequivocal cardiomyogenic potential [9–13]. Indeed, accumulating evidence suggests that PSC-CMs can survive, engraft, and form gap junctions between the host and graft [14, 15, 16••]. Most of the studies have shown functional benefits; however, several controversial points have been noted. In this review, we summarize the current progress and future perspectives of using PSC-CMs for treating heart failure.

This article is part of the Topical Collection on *Regenerative Medicine*

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Approaches for Cardiac Regeneration

Zebrafish, newt, and neonatal mice show heart regeneration after injury [17–19]. However, adult mammalian cardiomyocyte (CM) are terminally differentiated and have limited proliferative capacity. Thus, injured heart tissue is replaced by fibrous scar tissue, which causes contraction reduction and heart failure [20, 21]. The strategy to rescue the failing heart highlighted in this review involves replacing dead CMs with new CMs derived from PSCs. Human CMs are known to continue to proliferate postnatally but with a meager turnover rate (< 1% per year) in the adult heart [22]. Therefore, enhancement of proliferation in adult CMs is another attractive strategy for cardiac regeneration, as the mechanisms of proliferation are actively being investigated [23–28]. Strategy to induce endogenous CM-proliferation has been described in detail in other reviews [29–31].

Generation of PSC-CMs

CMs can be differentiated from PSCs by many protocols, mostly including two different culture methods; adherent [10, 11, 32, 33] and/or suspension culture [12, 13, 34]. In either approach, treatment with growth factors and/or small molecules, such as Wnt modulators, bone morphogenetic protein-4 (BMP-4), and activin A at precise time and dose is important [35], for recapitulating cardiac development [36]. Usually, at approximately 10 days after differentiation, cells start beating. Although differentiation efficiency improved dramatically in this 10 years, elimination of undifferentiated cells is essential for clinical application. There are many surface markers available to purify CM [37], while antibody-labeling is necessary. Glucose and/or glutamine depletion with lactate supplement can also purify CMs metabolically [38, 39].

Characteristics of PSC-CMs

PSC-CMs can contract, generate force, and propagate excitation; however, they show characteristics of fetal CMs in morphological, functional, and transcriptional analyses [40, 41]. Researchers have been able to miniaturize cardiac diseases on a dish, using patient-induced PSCs, such as channelopathy [42–44], cardiomyopathy [45–47], and cardiometabolic disorders [48]. Fetal phenotype is good enough to use for disease modeling and drug testing; however, it should be noted that human PSC-CMs are immature compared to adult phenotype. Several methods have been reported to enhance the maturity of PSC-CMs in vitro, for example, electrical [49] and mechanical stimulation [50], chemical treatment [51], and long-term culture [52–54]. The most advanced way to enhance the maturation of the PSC-CMs is the combination of these stimulations and three-dimensional tissue culture [55••]. However, to our knowledge, no report has shown that human PSC-CMs are identical to the adult phenotype in vitro.

PSC-CMs mature after transplantation morphologically in cell size, cell-cell junctions, sarcomere, and mitochondrial structures. They have many cues from the host tissue to mature [40]. They showed gradual time-dependent maturation after transplantation. Interestingly, when human PSC-CMs were transplanted to rat hearts, they did not mature even after 3 months [56]. On the other hand, mouse iPSC-CMs or rat primary CMs matured completely in rat hearts after transplantation [57]. Since the electrophysiological properties are quite different between human and rodent CMs; there may be a barrier to complete maturation of human CMs in rodent hearts. Alternatively, it could be possible that 3 months was too short for full mature to take place. Unlike transplant into rodent hearts, when human PSC-CMs were transplanted to non-human primates, the engrafted cells show evidence of maturation after 3 months [14]. This suggests the salubrious effects of heart environment in closely related species.

Preclinical Transplantation Studies of PSC-CMs

There are two different ways of transplantation method; dispersed cells or tissue-engineered cells. Both approaches have their pros and cons. Cell suspension is easy to prepare, cryopreserve, and inject directly to the inner muscle layer of the heart [14, 15, 16••, 58]. However, suspended cells leak out from injected sites easily, and most of the injected cells cannot sustain in the host heart, especially at the early stage after transplantation [59]. In contrast, tissue-engineered cells are easy to attach to the outer layer of the heart. Tissue-engineered cells can retain and survive, thereby reducing cell loss immediately after transplantation [60–62]. The most significant difference between these two transplantation methods is host-graft electrical integration. A study by Gerbin et al. has shown host-graft electrical integration by dispersed cells or micro-tissue particles, but not cardiac patches, and compared these methods by transplanting Ca^{2+} indicator GCaMP-tagged PSC-CMs to rat hearts [63]. Engineered PSC-CMs patch engrafted onto the epicardial surface have shown electrical coupling in guinea pig hearts; however, there is substantial scar tissue in between host and graft [62]. Therefore, dispersed cells or micro-tissue particles can contact directly to develop gap junctions between host and graft (Fig. 1). In other words, dispersed cells are easy to become a substrate of arrhythmia, if injected cells have different electrophysiological phenotypes, due to the direct contact between host and graft. Table 1 shows a summary of recent preclinical animal studies.

So far, in most studies, approximately 20-day-old PSC-CMs were transplanted to animal models. Funakoshi et al. have revealed that 20 days is the optimal timing for transplantation by comparing bioluminescent intensity from luciferase-tagged PSC-CMs in mouse-infarcted hearts [68].

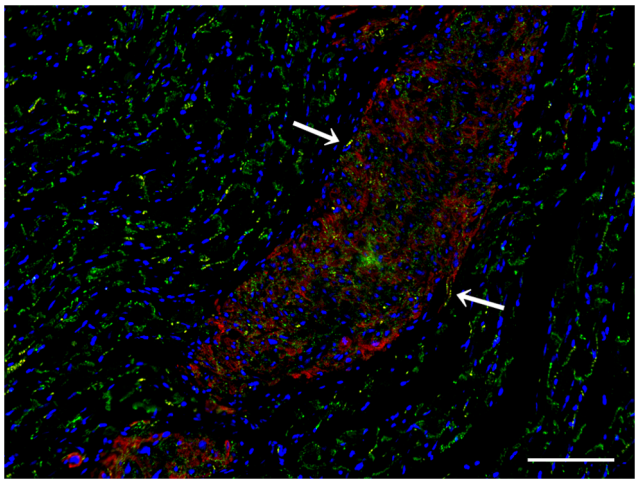

Fig. 1 Human iPSC-CMs graft in rat heart. Injected human induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) survived, engrafted, and formed gap junctions connecting with the host rat heart (arrows). Connexin-43 in *green*, beta-myosin heavy chain in *red* and DAPI in *blue*. Scale bar = 100 μm

Additionally, in rat hearts, graft size of 5-day-old cardiac progenitors is almost the same as PSC-CMs [56], although premature cells were thought to be more proliferative than 20-day-old CMs. These studies suggest that in addition to the proliferative potential, cell adhesion, and inflammatory response also determine engraftment of PSC-CMs. Further research is required to unveil the molecular mechanism of interaction between host and graft to enhance engraftment of PSC-CMs.

Most studies have used the acute or subacute model to treat PSC-CMs. However, the acute phase in the clinical setting is hard to treat by PSC-CMs, due to the time-consuming preparation of the cells. On the other hand, chronic ischemic heart failure may represent the more likely clinical setting for therapy using PSC-CMs. However, in a rat model of chronic heart failure at 1 month after myocardial infarction, PSC-CMs transplantation showed no functional benefit [65]. In a guinea pig chronic model, no electrical coupling was observed between the host and graft tissue, while electrical coupling between PSC-CMs and endogenous CMs was detected in more than half of acute model [67]. Scar tissue separated host and graft by histological analysis in both studies; thus, inflammation and fibrosis could make different response between host and graft in chronic heart disease.

Thus far, almost all previous reported studies discuss the effect of PSC-CMs on ischemic heart disease. Similar to the chronic model, the large animal non-ischemic model is challenging to develop, but the ischemic model is easy to reproduce by occluding coronary flow. Non-ischemic cardiomyopathy, such as dilated cardiomyopathy in advanced heart failure patients, is quite common [69]. Several gene mutations, such as *TTN*, *MYH7*, and *MYBPC3*, have been shown to cause non-ischemic cardiomyopathy [70]. Therefore, we need to develop large animal genetic models and show the feasibility of using PSC-CMs for treating non-ischemic cardiomyopathy.

Remaining Hurdles for Clinical Application

Post-Transplant Arrhythmias

When PSC-CMs transplanted to small animals such as a mouse, rat [64], and guinea pig [66], no post-transplant arrhythmias were observed; however, in non-human primates, post-transplant arrhythmias, such as ventricular tachycardia, were noticed [14, 15]. In the small animal models, heart rates

Table 1 Summary of preclinical animal studies using PSC-CM

Year	Animal	Cell	Cell number ($\times 10^6$)	Time point days after MI	Method	Duration (week)	LV function	Arrhythmia	Reference
2007	Rat	hESC-CM	10	4	Dispersed	4	Improved	–	[64]
2010	Rat	hESC-CM	10	28	Dispersed	12	No change	–	[65]
2012	Guinea pig	hESC-CM	100	10	Dispersed	4	Improved	–	[66]
2012	Swine	hiPSC-CM	25	28	Tissue	8	Improved	–	[60]
2014	Rat	hiPSC-CM[a]	2	7	Tissue	8	Improved	–	[61]
2014	Guinea pig	hESC-CM	80	28	Dispersed	4	No change	–	[67]
2014	NHP	hESC-CM	1000	14	Dispersed	12	No change	+	[14]
2014	Swine	hiPSC-CM[a]	1.1	7	Dispersed	4	Improved	–	[58]
2016	NHP	NHP iPSC-CM	400	14	Dispersed	12	Improved	+	[15]
2016	Guinea pig	hiPSC-CM[a]	7	7	Tissue	4	Improved	–	[62]
2018	NHP	hESC-CM	750	14	Dispersed	12	Improved	+	[16••]

NHP non-human primates, *ESC-CM* Embryonic stem cell-derived cardiomyocyte, *iPSC-CM* induced pluripotent stem cell-derived cardiomyocyte, *MI* myocardial infarction

[a] Include non-cardiomyocytes such as endothelial cells

are much faster than transplanted PSC-CMs; therefore, ectopic rhythms were masked. Recently Liu et al. showed that the cause of arrhythmias is ectopic pacing from transplanted PSC-CMs in hearts of non-human primates [16••]. Most of the reported arrhythmias were transient; thus, electrophysiological phenotype of engrafted PSC-CMs gradually matured to be less focally excitable after transplantation. In-depth knowledge of the methods is essential to enhance electrophysiological maturation in vitro before transplantation to prevent post-transplant arrhythmias.

Automaticity

PSC-CMs display nodal, atrial, and ventricular subtypes by the usual differentiation mechanism. Most reported studies have used the mixture of these subtypes as PSC-CMs for transplantation, although they show a different electrophysiological phenotype. Monolayer culture and metabolic selection induce the ventricular subtype [39, 71], whereas retinoic acid treatment induces the atrial subtype [72, 73]. The nodal cell is hardest to differentiate among the three subtypes, but pacemaker purification could provide the development of biological pacemaker. More than 15 years ago, Kehat et al. showed a pacemaking capacity of ESC-CMs without purification after transplantation to swine atrioventricular block model [74]. One of the hurdles preventing pacemaker cell differentiation is the limited number of specific markers for nodal cells [37]. Protze et al. has shown that loss of *NKX2–5* is a novel marker to purify nodal cells, especially sinoatrial nodes [75]. They clearly showed the pacemaking capacity of PSC-CMs after transplantation to rat atrioventricular block model; however, a proof-of-concept study in a large animal model is needed. Recently, another group showed *NKX2–5* negative *TBX5* positive subpopulation become nodal cells once differentiated into epicardial lineage [76].

Immune Rejection

Engraftment of human PSC-CMs in the primate heart requires immune suppression with a drug regimen that includes methylprednisolone, cyclosporine, and CTLA4-immunoglobulin [14, 16••]. MHC-matched primate PSC-CMs can engraft in the primate heart with only methylprednisolone and tacrolimus without anti-CTLA4 antibody, while MHC-mismatched PSC-CMs are rejected in primate heart with the same regimen [15]. It appears that long-term immune suppression will be necessary after transplantation even when HLA-matched PSC-CMs are used. To avoid long-term immune suppression, autologous or hypoimmunogenic PSC-CMs will be necessary. Because of the excessive cost associated with the generation and quality control of autologous iPSC-CMs, this approach will unlikely be adopted widely as the therapeutic strategy for the treatment of heart failure. On the other hand, hypoimmunogenic PSC derivative would be applicable for all patients [77–80], if the benefits were proved in a large animal study.

Clinical Trial

In France, human ESC-derived cardiovascular progenitors embedded in fibrin patch have been transplanted epicardially at the same time as coronary artery bypass surgery [81]. Cardiovascular progenitor cells were differentiated by 4-day exposure to BMP-2 and a fibroblast growth factor receptor-specific tyrosine kinase inhibitor, SU-5402. Progenitors were immunomagnetically sorted for a positive expression of SSEA-1 (a marker for loss of pluripotency). Five to ten million progenitor cells were introduced into each patch and sutured onto the epicardial surface over the infarct area. Six patients with chronic ischemic heart disease were enrolled. Since these were allogenic human ESC-derived cells, the patients were required to also receive immunosuppressive regimen including cyclosporine and mycophenolate mofetil for 1–2 months after surgery. All patients had uneventful postoperative course, except one patient died shortly after the operation. No tumor was detected, and none of the 5 operative survivors has experienced ventricular arrhythmias. All patients were reported to have improved symptomatically, although the relative contribution of cell therapy vs coronary artery bypass grafting to the clinical benefit is unclear. Furthermore, it is unclear whether how many, if any, of the introduced cells have survived once the immunosuppressive treatment has completed. Future studies will be needed to clarify the engraftment efficiency and treatment effect of this cardiovascular progenitor cell therapy.

Conclusions

In this review, we summarized the contemporary state and potential of PSC-CMs for treating heart failure, from a regenerative point of view. It is quite encouraging that a large number of cardiac cells can be obtained from PSCs, and that PSC-CMs can engraft and remuscularize infarcted non-human primate hearts. While these results are quite promising, more studies will be needed to address the translational challenges as discussed above. We anticipate phase I PSC-CMs clinical trial to start in the near future and take us one step closer to the emergence of the PSC-CM as durable therapy for ischemic heart diseases.

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Compliance with Ethical Standards

Conflict of Interest Shin Kadota and Yuji Shiba declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent All reported studies/experiments with animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

References

Papers of particular interest, published recently, have been highlighted as:
•• Of major importance

1. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and Stroke Statistics-2019 update: a report from the American Heart Association. Circulation. 2019;139:e56–e528.
2. Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet. 2004;364:141–8.
3. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. N Engl J Med. 2006;355:1199–209.
4. Bartunek J, Behfar A, Dolatabadi D, Vanderheyden M, Ostojic M, Dens J, et al. Cardiopoietic stem cell therapy in heart failure: the C-CURE (cardiopoietic stem cell therapy in heart failURE) multicenter randomized trial with lineage-specified biologics. J Am Coll Cardiol. 2013;61:2329–38.
5. Menasche P, Alfieri O, Janssens S, McKenna W, Reichenspurner H, Trinquart L, et al. The myoblast autologous grafting in ischemic cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. Circulation. 2008;117:1189–200.
6. Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, et al. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. Lancet. 2011;378:1847–57.
7. Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, et al. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction [CADUCEUS]: a prospective, randomised phase 1 trial. Lancet. 2012;379:895–904.
8. Hu X, Xu Y, Zhong Z, Wu Y, Zhao J, Wang Y, et al. A large-scale investigation of hypoxia-preconditioned allogeneic mesenchymal stem cells for myocardial repair in nonhuman primates: paracrine activity without remuscularization. Circ Res. 2016;118:970–83.
9. Laflamme MA, Murry CE. Heart regeneration. Nature. 2011;473:326–35.
10. Burridge PW, Matsa E, Shukla P, Lin ZC, Churko JM, Ebert AD, et al. Chemically defined generation of human cardiomyocytes. Nat Methods. 2014;11:855–60.
11. Lian X, Bao X, Zilberter M, Westman M, Fisahn A, Hsiao C, et al. Chemically defined, albumin-free human cardiomyocyte generation. Nat Methods. 2015;12:595–6.
12. Kempf H, Kropp C, Olmer R, Martin U, Zweigerdt R. Cardiac differentiation of human pluripotent stem cells in scalable suspension culture. Nat Protoc. 2015;10:1345–61.
13. Chen VC, Ye J, Shukla P, Hua G, Chen D, Lin Z, et al. Development of a scalable suspension culture for cardiac differentiation from human pluripotent stem cells. Stem Cell Res. 2015;15:365–75.
14. Chong JJ, Yang X, Don CW, Minami E, Liu YW, Weyers JJ, et al. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. Nature. 2014;510:273–7.
15. Shiba Y, Gomibuchi T, Seto T, Wada Y, Ichimura H, Tanaka Y, et al. Allogeneic transplantation of iPS cell-derived cardiomyocytes regenerates primate hearts. Nature. 2016;538:388–91.
16.•• Liu YW, Chen B, Yang X, Fugate JA, Kalucki FA, Futakuchi-Tsuchida A, et al. Human embryonic stem cell-derived cardiomyocytes restore function in infarcted hearts of non-human primates. Nat Biotechnol. 2018;36:597–605 **This article provides the evidence that post-transplant arrhythmias is due to abnormal impulse generation from engrafted human ESC-CMs acting as an ectopic pacemaker**.
17. Poss KD, Wilson LG, Keating MT. Heart regeneration in zebrafish. Science. 2002;298:2188–90.
18. Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, et al. Transient regenerative potential of the neonatal mouse heart. Science. 2011;331:1078–80.
19. Singh BN, Koyano-Nakagawa N, Gong W, Moskowitz IP, Weaver CV, Braunlin E, et al. A conserved HH-Gli1-Mycn network regulates heart regeneration from newt to human. Nat Commun. 2018;9:4237.
20. Laflamme MA, Murry CE. Regenerating the heart. Nat Biotechnol. 2005;23:845–56.
21. Tzahor E, Poss KD. Cardiac regeneration strategies: staying young at heart. Science. 2017;356:1035–9.
22. Bergmann O, Zdunek S, Felker A, Salehpour M, Alkass K, Bernard S, et al. Dynamics of cell generation and turnover in the human heart. Cell. 2015;161:1566–75.
23. Kimura W, Xiao F, Canseco DC, Muralidhar S, Thet S, Zhang HM, et al. Hypoxia fate mapping identifies cycling cardiomyocytes in the adult heart. Nature. 2015;523:226–30.
24. Wei K, Serpooshan V, Hurtado C, Diez-Cunado M, Zhao M, Maruyama S, et al. Epicardial FSTL1 reconstitution regenerates the adult mammalian heart. Nature. 2015;525:479–85.
25. Bassat E, Mutlak YE, Genzelinakh A, Shadrin IY, Baruch Umansky K, Yifa O, et al. The extracellular matrix protein agrin promotes heart regeneration in mice. Nature. 2017;547:179–84.
26. Morikawa Y, Heallen T, Leach J, Xiao Y, Martin JF. Dystrophin-glycoprotein complex sequesters yap to inhibit cardiomyocyte proliferation. Nature. 2017;547:227–31.
27. Mohamed TMA, Ang YS, Radzinsky E, Zhou P, Huang Y, Elfenbein A, et al. Regulation of cell cycle to stimulate adult cardiomyocyte proliferation and cardiac regeneration. Cell. 2018;173:104–16.
28. Hirose K, Payumo AY, Cutie S, Hoang A, Zhang H, Guyot R, et al. Evidence for hormonal control of heart regenerative capacity during endothermy acquisition. Science. 2019;364:184–8.
29. Sharma A, Zhang Y, Wu SM. Harnessing the induction of cardiomyocyte proliferation for cardiac regenerative medicine. Curr Treat Options Cardiovasc Med. 2015;17:404.
30. Lazar E, Sadek HA, Bergmann O. Cardiomyocyte renewal in the human heart: insights from the fall-out. Eur Heart J. 2017;38:2333–42.
31. Foglia MJ, Poss KD. Building and re-building the heart by cardiomyocyte proliferation. Development. 2016;143:729–40.

32. Lian X, Hsiao C, Wilson G, Zhu K, Hazeltine LB, Azarin SM, et al. Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. Proc Natl Acad Sci U S A. 2012;109:E1848–57.
33. Minami I, Yamada K, Otsuji TG, Yamamoto T, Shen Y, Otsuka S, et al. A small molecule that promotes cardiac differentiation of human pluripotent stem cells under defined, cytokine- and xeno-free conditions. Cell Rep. 2012;2:1448–60.
34. Yang L, Soonpaa MH, Adler ED, Roepke TK, Kattman SJ, Kennedy M, et al. Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population. Nature. 2008;453:524–8.
35. Friedman CE, Nguyen Q, Lukowski SW, Helfer A, Chiu HS, Miklas J, et al. Single-cell transcriptomic analysis of cardiac differentiation from human PSCs reveals HOPX-dependent cardiomyocyte maturation. Cell Stem Cell. 2018;23:586–98.
36. Galdos FX, Guo Y, Paige SL, VanDusen NJ, Wu SM, Pu WT. Cardiac regeneration: lessons from development. Circ Res. 2017;120:941–59.
37. Skelton RJP, Kamp TJ, Elliott DA, Ardehali R. Biomarkers of human pluripotent stem cell-derived cardiac lineages. Trends Mol Med. 2017;23:651–68.
38. Tohyama S, Hattori F, Sano M, Hishiki T, Nagahata Y, Matsuura T, et al. Distinct metabolic flow enables large-scale purification of mouse and human pluripotent stem cell-derived cardiomyocytes. Cell Stem Cell. 2013;12:127–37.
39. Tohyama S, Fujita J, Hishiki T, Matsuura T, Hattori F, Ohno R, et al. Glutamine oxidation is indispensable for survival of human pluripotent stem cells. Cell Metab. 2016;23:663–74.
40. Yang X, Pabon L, Murry CE. Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. Circ Res. 2014;114:511–23.
41. van den Berg CW, Okawa S, Chuva de Sousa Lopes SM, van Iperen L, Passier R, Braam SR, et al. Transcriptome of human foetal heart compared with cardiomyocytes from pluripotent stem cells. Development. 2015;142:3231–8.
42. Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flugel L, et al. Patient-specific induced pluripotent stem-cell models for long-QT syndrome. N Engl J Med. 2010;363:1397–409.
43. Itzhaki I, Maizels L, Huber I, Zwi-Dantsis L, Caspi O, Winterstern A, et al. Modelling the long QT syndrome with induced pluripotent stem cells. Nature. 2011;471:225–9.
44. Yazawa M, Hsueh B, Jia X, Pasca AM, Bernstein JA, Hallmayer J, et al. Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. Nature. 2011;471:230–4.
45. Ma D, Wei H, Lu J, Ho S, Zhang G, Sun X, et al. Generation of patient-specific induced pluripotent stem cell-derived cardiomyocytes as a cellular model of arrhythmogenic right ventricular cardiomyopathy. Eur Heart J. 2013;34:1122–33.
46. Sun N, Yazawa M, Liu J, Han L, Sanchez-Freire V, Abilez OJ, et al. Patient-specific induced pluripotent stem cells as a model for familial dilated cardiomyopathy. Sci Transl Med. 2012;4:130ra47.
47. Lan F, Lee AS, Liang P, Sanchez-Freire V, Nguyen PK, Wang L, et al. Abnormal calcium handling properties underlie familial hypertrophic cardiomyopathy pathology in patient-specific induced pluripotent stem cells. Cell Stem Cell. 2013;12:101–13.
48. Bellin M, Mummery CL. Inherited heart disease - what can we expect from the second decade of human iPS cell research? FEBS Lett. 2016;590:2482–93.
49. Nunes SS, Miklas JW, Liu J, Aschar-Sobbi R, Xiao Y, Zhang B, et al. Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. Nat Methods. 2013;10:781–7.
50. Ruan JL, Tulloch NL, Razumova MV, Saiget M, Muskheli V, Pabon L, et al. Mechanical stress conditioning and electrical stimulation promote contractility and force maturation of induced pluripotent stem cell-derived human cardiac tissue. Circulation. 2016;134:1557–67.
51. Parikh SS, Blackwell DJ, Gomez-Hurtado N, Frisk M, Wang L, Kim K, et al. Thyroid and glucocorticoid hormones promote functional T-tubule development in human-induced pluripotent stem cell-derived cardiomyocytes. Circ Res. 2017;121:1323–30.
52. Kamakura T, Makiyama T, Sasaki K, Yoshida Y, Wuriyanghai Y, Chen J, et al. Ultrastructural maturation of human-induced pluripotent stem cell-derived cardiomyocytes in a long-term culture. Circ J. 2013;77:1307–14.
53. Lundy SD, Zhu WZ, Regnier M, Laflamme MA. Structural and functional maturation of cardiomyocytes derived from human pluripotent stem cells. Stem Cells Dev. 2013;22:1991–2002.
54. Kadota S, Minami I, Morone N, Heuser JE, Agladze K, Nakatsuji N. Development of a reentrant arrhythmia model in human pluripotent stem cell-derived cardiac cell sheets. Eur Heart J. 2013;34:1147–56.
55.•• Ronaldson-Bouchard K, Ma SP, Yeager K, Chen T, Song L, Sirabella D, et al. Advanced maturation of human cardiac tissue grown from pluripotent stem cells. Nature. 2018;556:239–43 **This article provides the advanced way to enhance maturation of human PSC-CMs after only four weeks of culture *in vitro*.**
56. Kadota S, Pabon L, Reinecke H, Murry CE. In vivo maturation of human induced pluripotent stem cell-derived cardiomyocytes in neonatal and adult rat hearts. Stem Cell Reports. 2017;8:278–89.
57. Cho GS, Lee DI, Tampakakis E, Murphy S, Andersen P, Uosaki H, et al. Neonatal transplantation confers maturation of PSC-derived cardiomyocytes conducive to modeling cardiomyopathy. Cell Rep. 2017;18:571–82.
58. Ye L, Chang YH, Xiong Q, Zhang P, Zhang L, Somasundaram P, et al. Cardiac repair in a porcine model of acute myocardial infarction with human induced pluripotent stem cell-derived cardiovascular cells. Cell Stem Cell. 2014;15:750–61.
59. Zhang M, Methot D, Poppa V, Fujio Y, Walsh K, Murry CE. Cardiomyocyte grafting for cardiac repair: graft cell death and anti-death strategies. J Mol Cell Cardiol. 2001;33:907–21.
60. Kawamura M, Miyagawa S, Miki K, Saito A, Fukushima S, Higuchi T, et al. Feasibility, safety, and therapeutic efficacy of human induced pluripotent stem cell-derived cardiomyocyte sheets in a porcine ischemic cardiomyopathy model. Circulation. 2012;126:S29–37.
61. Masumoto H, Ikuno T, Takeda M, Fukushima H, Marui A, Katayama S, et al. Human iPS cell-engineered cardiac tissue sheets with cardiomyocytes and vascular cells for cardiac regeneration. Sci Rep. 2014;4:6716.
62. Weinberger F, Breckwoldt K, Pecha S, Kelly A, Geertz B, Starbatty J, et al. Cardiac repair in guinea pigs with human engineered heart tissue from induced pluripotent stem cells. Sci Transl Med. 2016;8:363ra148.
63. Gerbin KA, Yang X, Murry CE, Coulombe KL. Enhanced electrical integration of engineered human myocardium via intramyocardial versus epicardial delivery in infarcted rat hearts. PLoS One. 2015;10:e0131446.
64. Laflamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK, et al. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat Biotechnol. 2007;25:1015–24.
65. Fernandes S, Naumova AV, Zhu WZ, Laflamme MA, Gold J, Murry CE. Human embryonic stem cell-derived cardiomyocytes engraft but do not alter cardiac remodeling after chronic infarction in rats. J Mol Cell Cardiol. 2010;49:941–9.
66. Shiba Y, Fernandes S, Zhu WZ, Filice D, Muskheli V, Kim J, et al. Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. Nature. 2012;489:322–5.
67. Shiba Y, Filice D, Fernandes S, Minami E, Dupras SK, Biber BV, et al. Electrical integration of human embryonic stem cell-derived

cardiomyocytes in a guinea pig chronic infarct model. J Cardiovasc Pharmacol Ther. 2014;19:368–81.
68. Funakoshi S, Miki K, Takaki T, Okubo C, Hatani T, Chonabayashi K, et al. Enhanced engraftment, proliferation, and therapeutic potential in heart using optimized human iPSC-derived cardiomyocytes. Sci Rep. 2016;6:19111.
69. Bozkurt B, Colvin M, Cook J, Cooper LT, Deswal A, Fonarow GC, et al. Current diagnostic and treatment strategies for specific dilated cardiomyopathies: a scientific statement from the American Heart Association. Circulation. 2016;134:e579–646.
70. McNally EM, Barefield DY, Puckelwartz MJ. The genetic landscape of cardiomyopathy and its role in heart failure. Cell Metab. 2015;21:174–82.
71. Cyganek L, Tiburcy M, Sekeres K, Gerstenberg K, Bohnenberger H, Lenz C, et al. Deep phenotyping of human induced pluripotent stem cell-derived atrial and ventricular cardiomyocytes. JCI Insight. 2018;3:e99941.
72. Devalla HD, Schwach V, Ford JW, Milnes JT, El-Haou S, Jackson C, et al. Atrial-like cardiomyocytes from human pluripotent stem cells are a robust preclinical model for assessing atrial-selective pharmacology. EMBO Mol Med. 2015;7:394–410.
73. Lee JH, Protze SI, Laksman Z, Backx PH, Keller GM. Human pluripotent stem cell-derived atrial and ventricular cardiomyocytes develop from distinct mesoderm populations. Cell Stem Cell. 2017;21:179–94.
74. Kehat I, Khimovich L, Caspi O, Gepstein A, Shofti R, Arbel G, et al. Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. Nat Biotechnol. 2004;22:1282–9.
75. Protze SI, Liu J, Nussinovitch U, Ohana L, Backx PH, Gepstein L, et al. Sinoatrial node cardiomyocytes derived from human pluripotent cells function as a biological pacemaker. Nat Biotechnol. 2017;35:56–68.
76. Zhang JZ, Termglinchan V, Shao NY, Itzhaki I, Liu C, Ma N, et al. A human iPSC double-reporter system enables purification of cardiac lineage subpopulations with distinct function and drug response profiles. Cell Stem Cell. 2019;24:802–811.e5. https://doi.org/10.1016/j.stem.2019.02.015.
77. Gornalusse GG, Hirata RK, Funk SE, Riolobos L, Lopes VS, Manske G, et al. HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. Nat Biotechnol. 2017;35:765–72.
78. Mattapally S, Pawlik KM, Fast VG, Zumaquero E, Lund FE, Randall TD, et al. Human leukocyte antigen class I and II knockout human induced pluripotent stem cell-derived cells: universal donor for cell therapy. J Am Heart Assoc. 2018;7:e010239.
79. Deuse T, Hu X, Gravina A, Wang D, Tediashvili G, De C, et al. Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. Nat Biotechnol. 2019;37:252–8.
80. Xu H, Wang B, Ono M, Kagita A, Fujii K, Sasakawa N, et al. Targeted disruption of HLA genes via CRISPR-Cas9 generates iPSCs with enhanced immune compatibility. Cell Stem Cell. 2019;24:566–78.
81. Menasche P, Vanneaux V, Hagege A, Bel A, Cholley B, Parouchev A, et al. Transplantation of human embryonic stem cell-derived cardiovascular progenitors for severe ischemic left ventricular dysfunction. J Am Coll Cardiol. 2018;71:429–38.