Inflammation and Tissue Remodeling as Potential Therapeutic Targets

Review

Therapeutic Potential of Pluripotent Stem Cells for Cardiac Repair after Myocardial Infarction

Satomi Okano^{a,b} and Yuji Shiba^{*,a,b,c}

^aDepartment of Regenerative Science and Medicine, Shinshu University; 3–1–1 Asahi, Matsumoto, Nagano 390–8621, Japan; ^bInstitute for Biomedical Sciences, Shinshu University; 3–1–1 Asahi, Matsumoto, Nagano 390–8621, Japan; and ^cDepartment of Cardiovascular Medicine, Shinshu University; 3–1–1 Asahi, Matsumoto, Nagano 390–8621, Japan.

Received March 30, 2018

Myocardial infarction occurs as a result of acute arteriosclerotic plaque rupture in the coronary artery, triggering strong inflammatory responses. The necrotic cardiomyocytes are gradually replaced with non-contractile scar tissue that eventually manifests as heart failure. Pluripotent stem cells (PSCs) show great promise for widespread clinical applications, particularly for tissue regeneration, and are being actively studied around the world to help elucidate disease mechanisms and in the development of new drugs. Human induced PSCs also show potential for regeneration of the myocardial tissue in experiments with small animals and in *in vitro* studies. Although emerging evidence points to the effectiveness of these stem cell-derived cardiomyocytes in cardiac regeneration, several challenges remain before clinical application can become a reality. Here, we provide an overview of the present state of PSC-based heart regeneration and highlight the remaining hurdles, with a particular focus on graft survival, immunogenicity, posttransplant arrhythmia, maintained function, and tumor formation. Rapid progress in this field along with advances in biotechnology are expected to resolve these issues, which will require international collaboration and standardization.

Key words pluripotent stem cell; cardiomyocyte; heart regeneration

1. INTRODUCTION

Heart disease is one of the leading causes of death worldwide.¹⁾ Cardiomyocytes are terminally differentiated cells; however, their poor reusability has made it extremely difficult to restore injured cardiac function using currently available therapies. Approximately four billion cardiomyocytes are present in the heart of an adult human, and around one billion are lost during a typical myocardial infarction.²⁾ Currently, heart transplantation is the only effective treatment for severe heart failure, but a donor shortage remains a serious problem. During the progress of myocardial infarction, neutrophil infiltration occurs within 24 h after coronary artery occlusion, followed by recruitment of monocytes with a peak at 72 h.³⁾ The formation of granulation tissue takes place simultaneously with the removal of dead tissue, and progressively resulting in the development of scar tissue.⁴⁾ Regenerative therapies using somatic stem cells, including bone marrow-derived cells,⁵⁾ mesenchymal stem cells,^{6–8)} and cardiomyocyte stem cells,⁹⁾ have been performed to treat infarcted hearts. When bone marrow-derived mononuclear cells are transplanted, an angiogenesis effect is observed based on an increase in the secretion of inflammatory cytokines such as interleukin (IL)-1 β and tumor necrosis factor- α .¹⁰⁾ However, these somatic stem cells have poor ability to differentiate into cardiomyocytes and cannot remain for long term after transplantation.¹¹⁾ These cell therapeutic effects are thought to be due to humoral factors secreted by the transplanted cells to exert a so-called paracrine effect.¹²⁾ Another recently emerging strategy for myocar-

dial regeneration treatment is through the use of pluripotent stem cells (PSCs). PSCs are an ideal tool for regeneration of the heart, which requires a large number of cardiomyocytes. They have the advantages of nearly endless proliferative capacity and the ability to differentiate into almost all cell types, including cardiomyocytes. Here, we describe the recent progress in myocardial regeneration therapy using PSCs and address future tasks to allow its clinical application.

2. ESTABLISHMENT OF PSCs

In 1981, Evans and Kaufman succeeded in isolating PSCs directly from delayed mouse embryos¹³⁾; the cells were originally named EK cells after the scientists' names, but are now more commonly known as embryonic stem cells (ESCs). These ESCs are karyotypically normal, able to induce teratocarcinoma when injected into syngeneic mice, and able to form embryoid bodies (EBs) in culture. Within 1 month of the first report, Martin¹⁴⁾ described the isolation of mouse ESCs from an inner cell mass derived from mouse blastocysts. Following the success of establishing mouse ESC lines, Thomson *et al.*¹⁵⁾ reported in 1998 that human ESCs could be established from blastocysts. Human ESCs have since generated substantial interest as a good candidate source for tissue regeneration. However, the use of human ESCs is laden with ethical concerns, which has restricted ESC research and clinical applications.

In 2006, Takahashi and Yamanaka¹⁶⁾ introduced four factors (Oct3/4, Sox2, c-Myc, and Klf4) into murine fibroblasts *via*

* To whom correspondence should be addressed. e-mail: yshiba@shinshu-u.ac.jp

retroviral infection, which successfully directly reprogrammed differentiated adult somatic cells to the embryonic-like stage, and were designated as induced pluripotent stem cells (iPSCs). The following year, that group succeeded in generating iPSCs from human fibroblasts using the same four factors as applied to mouse iPSCs.¹⁷⁾ The emergence of iPSCs can avoid the most significant concerns related to the destruction of embryos required in ESC research since it only involves the genetic reprogramming of somatic cells. In addition, iPSCs have particular advantages related to immunological aspects that allow them to be used in autologous transplantations.

3. DIFFERENTIATION OF PSCs INTO CARDIOMYOCYTES

Mouse ESCs can be transdifferentiated into cardiomyocytes by forming three-dimensional cell aggregates in suspension culture conditions. These EBs then start beating spontaneously.¹⁸⁾ However, this kind of cardiac differentiation of human ESCs is rare, typically yielding <1% cardiomyocytes. In 2001, Kehat *et al.*¹⁹⁾ collected EBs after suspension culture of 10 d, then were replated on gelatin-coated plates and cultured with Dulbecco's modified Eagle's medium containing 20% fetal bovine serum to generate human ESC-derived cardiomyocytes. Through this modification, the cardiac differentiation efficiency was slightly enhanced, and almost 8% of all EBs showed a contractile property. In 2003, the Mummery research group first demonstrated the induction of cardiomyocyte differentiation in human ESCs that did not undergo as spontaneous cardiogenesis by co-culturing with END-2 cells, visceral endoderm-like cells derived from the mouse.²⁰⁾ They subsequently provided a protocol based on serum-free co-culture with END-2 cells or by exposing human ESC-conditioned medium of END-2 cells to generate approximately 25% cardiomyocytes.²¹⁾ To enhance the purity of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs), various protocols have been exploited that mimic the embryonic heart development environment. These protocols induce cardiomyocytes mainly by stimulating or suppressing three signal transductions, nodal/activin, bone morphogenic protein (BMP), and Wnt signaling, which are discussed in turn below.

3.1. Nodal/Activin Nodal is a member of the transforming growth factor-beta (TGF- β) family of growth factors and is an essential factor for the formation of the gastrulation and germ layer.²²⁾ In the absence of Nodal expression, no mesoderm is formed. Another protein in the TGF- β superfamily,

activin, also has strong mesoderm-inducing action. In 2007, Laflamme and colleagues demonstrated a method of myocardial differentiation using activin A as a substitute for Nodal.²³⁾

3.2. BMP Similar to Nodal and activin, BMP is a peptide belonging to the TGF- β superfamily but acts as a ventralization signal at the gastrulation invagination stage instead of having an effect on early mesoderm formation.²⁴⁾ *In vivo*, BMP was shown to suppress cardiogenesis on the early embryo²⁵⁾; thus, BMP signaling suppresses cardiomyocyte differentiation in the early stage and promotes differentiation in the later stage.

3.3. Wnt Signaling There are two Wnt signaling pathways: Canonical and noncanonical. Each pathway plays an important role in cardiogenesis. The canonical Wnt signaling pathway is essential for normal gastrulation and mesoderm formation in the early development of embryo.²⁶⁾ Like BMP, Wnt/ β -catenin signaling was reported to play a time-specific biphasic role in myocardial differentiation.²⁷⁾ However, in contrast to BMP signaling, early canonical Wnt signaling stimulates mesoderm induction, while inhibiting cardiogenesis at later time points.^{27–30)}

4. TRANSPLANTATION OF PSC-DERIVED CARDIOMYOCYTES

Preclinical studies conducted to date using PSC-CMs for cardiac repair are summarized in Table 1. Multiple studies involved the transplantation of hPSC-CMs as suspensions of dispersed cells into an infarcted myocardium. The Gepstein group³¹⁾ transplanted undifferentiated ESCs, ESC-CMs, or noncardiac ESC derivatives into an immunosuppressed rat heart that they previously induced a myocardial infarction. Interestingly, only recipients of hESC-CMs showed attenuation of the left ventricular remodeling process measured by echocardiography. Laflamme *et al.*²³⁾ transplanted hESC-CMs with cell-surviving factors including Matrigel into athymic rats, and confirmed a substantially large graft myocardium. They also showed that transplantation of hESC-CMs, unlike noncardiac derivatives, improved cardiac contractile function after myocardial infarction by cardiac magnetic resonance imaging. In addition, we previously reported that transplanted hESC-CMs into guinea pigs, with a heart rate that is relatively more matched to that of the human heart compared to other rodents, could electrically couple to the host hearts based on observation of the fluorescent calcium indicator, GCaMP.³²⁾ Collectively, those studies demonstrated that after transplanta-

Table 1. Preclinical Studies of Pluripotent Stem Cell-Derived Cardiomyocyte Transplantation

Year (reference)	Host animal	Cell type	Cell number (cells)	Time of Tx (post-MI)	Cell delivery method	Observation period (weeks)	Cardiac function	Post-Tx arrhythmia
2007 ³¹⁾	Rat	hESC-CMs	1.5×10^6	7–10 d	Intramyocardial direct injection	4	Improved	—
2007 ²³⁾	Rat	hESC-CMs	1×10^7	4 d	Intramyocardial direct injection	4	Improved	—
2012 ³²⁾	Guinea pig	hESC-CMs	1×10^8	10 d	Intramyocardial direct injection	4	Improved	—
2012 ³⁷⁾	Pig	hiPSC-CMs	2.5×10^7	28 d	Patch	8	Improved	—
2014 ³⁸⁾	Rat	hiPSC-CMs/ECs/MCs	2×10^6	7 d	Patch	8	Improved	—
2014 ⁴⁶⁾	Guinea pig	hESC-CMs	8×10^7	28 d	Intramyocardial direct injection	4	No change	—
2014 ³³⁾	Monkey	hESC-CMs	1×10^9	14 d	Intramyocardial direct injection	12	No change	+
2016 ³⁴⁾	Monkey	Monkey iPSC-CMs	4×10^8	14 d	Intramyocardial direct injection	12	Improved	+
2016 ³⁹⁾	Guinea pig	hESC-CMs	5×10^6	7 d	Patch	4	Improved	—

MI, myocardial infarction; Tx, transplantation; hESC, human embryonic stem cell; CMs, cardiomyocytes; hiPSC, human induced pluripotent stem cell; ECs, endothelial cells; MCs, vascular mural cells.

tion into small animal models, hESC-CMs improved cardiac function and they also described decreased incidence of post-transplant arrhythmia. In 2014, the Murry group³³⁾ transplanted 1×10^9 hESC-CMs into nonhuman primate hearts that resulted in long-term survival with all of the grafts becoming electrically integrated with the host heart. Unlike previous studies that used small animals, they observed posttransplant ventricular arrhythmia. Subsequently, our group³⁴⁾ performed allogeneic transplantation of nonhuman primate iPSC-CMs, which improved the contractile function of the infarcted heart but also significantly increased the incidence of ventricular arrhythmia. These evidences thus far suggest that PSC-CMs may cause posttransplant arrhythmia when transplanted into large animal models with a good match in both heart size and beating rate of the host and donor.

Despite the success in the engraftment and electrical coupling of PSC-CMs with the host myocardium, some doubts remain about the fact that a simple injection of dispersed cells alone will form an organized, highly aligned cardiac tissue structure. PSC-CM grafts are typically comprised of small, irregularly contoured islands of immature cardiomyocytes. Accordingly, substantial efforts have been made to form a cardiac tissue-like structure with tissue engineering strategies. Okano *et al.*³⁵⁾ developed a temperature-responsive culture dish, making it possible to collect cells in sheet form with retained structure and function in a noninvasive manner. Moreover, Sekine *et al.*³⁶⁾ reported that cell sheet transplantation yielded greater cell survival than cell injection. Kawamura *et al.*³⁷⁾ transplanted a hiPSC-CMs sheet to a porcine model of ischemic cardiomyopathy and observed graft survival and mechanical benefits. Masumoto *et al.*³⁸⁾ created cardiac tissue sheets consisting of hiPSC-CMs, endothelial cells, and vascular mural cells, which were transplanted into a rat model of myocardial infarction and showed improvement of cardiac contractile function. Weinberger *et al.*³⁹⁾ created human-engineered heart tissue (hEHT) derived from iPSC-CMs/endothelial cells and human endothelial cell patches (hEETs). After transplanting hEHT, hEETs, and cell-free patches onto the left ventricular wall of cryo-injured guinea pig hearts, only the hEHT showed remuscularization of the scar tissue and electrical coupling to the host heart, resulting an improvement of cardiac contractile function. However, the Murry group reported that tissue-engineered cardiac patches did not couple electrically with the host heart.⁴⁰⁾ Thus, electrical integration of a cardiac tissue-like structure transplanted into the heart needs further investigation.

Another possible strategy to deliver PSC-CMs is intracoronary injection of a cell suspension. Many studies have reported the injection of somatic stem cells such as bone marrow cells (BMCs) into the coronary arteries, and clinical trials are underway. In 2002, Strauer *et al.*⁴¹⁾ reported an improvement of cardiac function in 10 patients with myocardial infarction after intracoronary injection of autologous mononuclear BMCs. In the September 2006 issue from the *New England Journal of Medicine*, three papers were published related to the intracoronary administration of autologous BMCs in patients suffering from myocardial infarction. Two of them demonstrated improvement of cardiac function,^{42,43)} while the other one did not show any effect.⁴⁴⁾ In addition, intracoronary injection of mesenchymal stem cells performed after myocardial infarction was reported to improve both cardiac function

and coronary blood flow.⁴⁵⁾ But the effects of PSC-CMs transplanted by coronary injection on engraftment of an infarcted myocardium remain unexplored.

Accumulated evidences suggest that transplantation of PSC-CMs improves cardiac contractile function in an acute or subacute myocardial infarction model; however, limited studies have been conducted with a chronic myocardial infarction model. We did not find any mechanical benefits of hESC-CMs transplantation in a guinea pig model 4 weeks after cryo-injury of the heart compared to vehicle-treated animals.⁴⁶⁾ On the other hand, Kawamura *et al.*³⁷⁾ observed mechanical improvement in a porcine chronic myocardial infarction model. Therefore, further studies are required involving a chronic myocardial infarction model that reflects more accurately the patient population who are at the end-stage of heart failure.

5. CURRENT LIMITATIONS AND FUTURE TASKS

In 2015, the Menasche group reported the first clinical trial of the use of human ESCs in myocardial regeneration.⁴⁷⁾ They implanted 4×10^6 myocardial progenitor cells generated from ESCs into a fibrin patch and transplanted the epicardium with cardiac bypass surgery. Prior to surgery, as the patient received an implantable cardioverter defibrillator, no arrhythmia occurred after transplantation and tumor formation was not observed during 3 months following the operation. Thus, PSCs show good promise in myocardial regeneration therapy. Nevertheless, several problems remain to be addressed before their practical application in routine medical care.

5.1. Poor Survival of Transplanted Cardiomyocytes

Poor survival of grafted cells is one of the major factors hindering the therapeutic effects of cell transplantation.⁴⁸⁾ Therefore, the key to a successful regeneration treatment is to promote the efficient engraftment of transplanted cells. Here, we describe the three main factors that could prevent the survival of transplanted cardiomyocytes as potential targets for improvement: Inflammation; anoikis; and ischemia.

5.1.1. Inflammation

Myocardial infarction causes an intense inflammatory response.⁴⁹⁾ In the acute phase, once the myocardial cells are destroyed, they undergo phagocytosis and proteolysis to remove debris from the injured tissue. The infarcted myocardium activates various signaling pathways as Toll-like receptor signaling, resulting in the generation of reactive oxygen species that induce cytokine and chemokine upregulation. In the chronic phase, fibrosis in the infarcted region progresses, forming an extremely difficult environment for the survival of transplanted cells. Suzuki *et al.*⁴⁸⁾ reported that suppression of IL-1 β , an important proinflammatory cytokine, doubled the number of transplanted cells after 72h. Similarly, Murtuza *et al.*⁵⁰⁾ demonstrated that inhibition of IL-1 increased the survival rate of the transplanted cells after 3 weeks by more than 6-fold, accompanied by a decrease in cardiac hypertrophy and improvement in the left ventricular ejection fraction in the infarcted murine myocardium. Thus, inflammation control is one of the keys to a successful graft survival.

5.1.2. Anoikis

One of the main reasons of the death of graft cells is apoptosis by activation of the Jun N-terminal kinase pathway⁵¹⁾ triggered by the loss of matrix attachments, which is known as anoikis.⁵²⁾ Several cell scaffold materials such as Matri-

gel,²³⁾ hyaluronan acid-based extracellular matrix,^{53–55)} and thermosensitive and biodegradable hydrogels⁵⁶⁾ have been tested for their ability to control anoikis. Laflamme *et al.*²³⁾ reported that a pro-survival cocktail including Matrigel with hESC-CMs could significantly suppress anoikis and enhance graft survival. Moreover, Uesugi and colleagues discovered new low molecular-weight compounds with an anoikis-inhibitory effect.^{57,58)}

5.1.3. Ischemia

Zhang *et al.*⁵⁹⁾ revealed that ischemia was one of the main contributors to cell death after transplantation. They transplanted cardiomyocytes into vascularized 2-week-old cardiac granulation tissue or into a normal myocardium. They observed respectively 53 and 86% of reduction of cell death, compared to the acute necrotic myocardium, by measuring the terminal deoxynucleotide transferase deoxyuridine triphosphate (dUTP)-nick-end labeling staining (a marker of apoptosis). Consistent with that finding, activation and/or upregulation of heme oxygenase-1 was shown to reduce the infarct size and protect grafted cardiomyocytes from oxidative stress.^{60,61)} Additionally, heat-shock treatment improved cell tolerance to oxidative stress.^{62,63)}

5.2. Immunogenicity In most of the preclinical studies, human PSC-CMs were transplanted into genetically or drug-induced immunodeficient animal models^{33,34)}; however, few studies have evaluated the immune response in a more clinically relevant model. Presumably, a PSC-CMs graft can survive without immune rejection *via* the same protocol used in whole-heart transplantation, but this approach would increase the risks of infection and malignancy.⁶⁴⁾ Accordingly, substantial research has been carried out to reduce the use of immunosuppressants and avoid these risks. One possible strategy is now emerging through the banking of diverse human leukocyte antigen (HLA)-typed PSCs,⁶⁵⁾ which would allow for the matching of HLA types in allogeneic transplantation. Our group showed that major histocompatibility complex-matched allogeneic transplantation of nonhuman primate iPSC-CMs resulted in a long-term graft survival with relatively mild immunosuppression.³⁴⁾ In addition, recent progress in genome-editing technology has provided HLA knock-out PSC lines, which could also presumably prevent an HLA-induced immune response. Thus it is theoretically possible to create autologous cell-based therapies with iPSC technology, although the estimated costs required to create, test, and store these human iPSCs are currently prohibitive for the widespread application of this technique.

5.3. Tumor Formation Tumor formation is also a major concern of the application of PSCs in myocardial regeneration therapy, because cardiac regeneration requires a large number of cardiomyocytes differentiated from PSCs. If a cell population contains a certain proportion of undifferentiated cells, the risk of teratoma formation will increase along with the number of transplanted cells. Tumorigenesis after transplantation in an undifferentiated state has been reported for both ESCs⁶⁶⁾ and iPSCs.⁶⁷⁾ Although teratoma formation has not been reported in PSC-derived cardiomyocytes, it is necessary to establish a differentiation protocol capable of producing high-purity cardiomyocytes and to confirm their long-term safety after cell transplantation.

5.4. Arrhythmia Since arrhythmic events are the leading cause of death in patients with heart failure,⁶⁸⁾ a

careful attention should be paid to the possibility of post-transplant arrhythmia. There are several advantages^{32,69)} and disadvantages⁷⁰⁾ regarding posttransplant arrhythmia after transplantation of PSC-CMs in small animal models. As mentioned in the previous section, recent studies using large animal models have shown that transplantation of PSC-CMs innately induces ventricular arrhythmia. Although the underlying mechanisms remain unclear, it could be caused by reentry, automaticity, or triggered activity of PSC-CMs.⁷¹⁾ Thus, more studies are required to clarify these mechanisms to achieve control over posttransplant arrhythmia.

5.5. Immature Phenotype of PSC-Derived Cardiomyocytes Human iPSC-derived cardiomyocytes have depolarized maximum diastolic potentials,⁷²⁾ lack t-tubules,⁷³⁾ exhibit mitochondrial dysfunction,⁷⁴⁾ and have weak expression of the cardiac maturation marker cTnI even when cultured for 9 months *in vitro*.⁷⁵⁾ *In vivo*, although transplanted cardiomyocytes have been reported to mature to a certain extent,⁷⁶⁾ the grafted cardiomyocytes have a weaker expression of cardiac muscle-specific proteins than the host myocardial tissue and show a clearly distinct tissue structure. Furthermore, we reported that the expression of gap junction proteins such as connexin 43 was relatively weak in the graft myocardium, and there was nonuniformity of electrical conduction in the grafted myocardium as the heart rate increased.^{32,34)} Several reports suggested that this electrical heterogeneity was the main cause of posttransplant ventricular arrhythmia.^{34,76)}

6. CONCLUSION

Research on cardiac repair using PSCs has progressed rapidly in recent years owing to the hard work of multiple worldwide investigators, and the first clinical trial of ESCs is now ongoing.⁷⁷⁾ However, substantial challenges remain to be resolved before clinical application. For example, new technology for the manufacturing of iPSCs and the differentiation/purification of cardiomyocytes is required at a lower cost. Moreover, this research is currently being conducted at multiple independent institutions involving various protocols; therefore, more collaboration to establish a standardized protocol will be required for use in clinical trials.

Conflict of Interest S.O. is an employee of Myoridge, Inc. Y.S. has no conflict of interest.

REFERENCES

- Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delosantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE,

- Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabwejian J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasser K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA III, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De Leon FR, Rosenfeld LC, Rushon L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010. A systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, **380**, 2095–2128 (2012).
- 2) Laflamme MA, Murry CE. Heart regeneration. *Nature*, **473**, 326–335 (2011).
 - 3) Cheng B, Chen HC, Chou IW, Tang TW, Hsieh PC. Harnessing the early post-injury inflammatory responses for cardiac regeneration. *J. Biomed. Sci.*, **24**, 7 (2017).
 - 4) Laflamme MA, Murry CE. Regenerating the heart. *Nat. Biotechnol.*, **23**, 845–856 (2005).
 - 5) Moelker AD, Baks T, van den Bos EJ, van Geuns RJ, de Feyter PJ, Duncker DJ, van der Giessen WJ. Reduction in infarct size, but no functional improvement after bone marrow cell administration in a porcine model of reperfused myocardial infarction. *Eur. Heart J.*, **27**, 3057–3064 (2006).
 - 6) Amado LC, Saliaris AP, Schuleri KH, St John M, Xie JS, Cattaneo S, Durand DJ, Fitton T, Kuang JQ, Stewart G, Lehrke S, Baumgartner WW, Martin BJ, Heldman AW, Hare JM. Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. *Proc. Natl. Acad. Sci. U.S.A.*, **102**, 11474–11479 (2005).
 - 7) Dai W, Hale SL, Martin BJ, Kuang JQ, Dow JS, Wold LE, Kloner RA. Allogeneic mesenchymal stem cell transplantation in postinfarcted rat myocardium. Short- and long-term effects. *Circulation*, **112**, 214–223 (2005).
 - 8) Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, Hao H, Ishino K, Ishida H, Shimizu T, Kangawa K, Sano S, Okano T, Kitamura S, Mori H. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. *Nat. Med.*, **12**, 459–465 (2006).
 - 9) Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, Beache GM, Wagner SG, Leri A, Hosoda T, Sanada F, Elmore JB, Goichberg P, Cappetta D, Solankhi NK, Fahsah I, Rokosh DG, Slaughter MS, Kajstura J, Anversa P. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO). Initial results of a randomised phase 1 trial. *Lancet*, **378**, 1847–1857 (2011).
 - 10) Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, Masaki H, Mori Y, Iba O, Tateishi E, Kosaki A, Shintani S, Murohara T, Imaizumi T, Iwasaka T. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation*, **104**, 1046–1052 (2001).
 - 11) Hu X, Xu Y, Zhong Z, Wu Y, Zhao J, Wang Y, Cheng H, Kong M, Zhang F, Chen Q, Sun J, Li Q, Jin J, Li Q, Chen L, Wang C, Zhan H, Fan Y, Yang Q, Yu L, Wu R, Liang J, Zhu J, Wang Y, Jin Y, Lin Y, Yang F, Jia L, Zhu W, Chen J, Yu H, Zhang J, Wang J. A large-scale investigation of hypoxia-preconditioned allogeneic mesenchymal stem cells for myocardial repair in nonhuman primates. Paracrine activity without remuscularization. *Circ. Res.*, **118**, 970–983 (2016).
 - 12) Hodgkinson CP, Bareja A, Gomez JA, Dzau VJ. Emerging concepts in paracrine mechanisms in regenerative cardiovascular medicine and biology. *Circ. Res.*, **118**, 95–107 (2016).
 - 13) Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature*, **292**, 154–156 (1981).
 - 14) Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 7634–7638 (1981).
 - 15) Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science*, **282**, 1145–1147 (1998).
 - 16) Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, **126**, 663–676 (2006).
 - 17) Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, **131**, 861–872 (2007).
 - 18) Doetschman TC, Eistetter H, Katz M, Schmidt W, Kemler R. The *in vitro* development of blastocyst-derived embryonic stem cell lines. Formation of visceral yolk sac, blood islands and myocardium. *J. Embryol. Exp. Morphol.*, **87**, 27–45 (1985).
 - 19) Kehat I, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A, Livne E, Binah O, Itskovitz-Eldor J, Gepstein L. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J. Clin. Invest.*, **108**, 407–414 (2001).
 - 20) Mummery C, Ward-van Oostwaard D, Doevendans P, Spijker R, van den Brink S, Hassink R, van der Heyden M, Ophhof T, Pera M, de la Riviere AB, Passier R, Tertoolen L. Differentiation of human embryonic stem cells to cardiomyocytes. Role of coculture with visceral endoderm-like cells. *Circulation*, **107**, 2733–2740 (2003).
 - 21) Mummery CL, Ward D, Passier R. Differentiation of human embryonic stem cells to cardiomyocytes by coculture with endoderm in serum-free medium. *Curr. Protocols Stem Cell Biol.*, **Chapter 1**, Unit 1F.2 (2007).
 - 22) Gadue P, Huber TL, Nostro MC, Kattman S, Keller GM. Germ layer induction from embryonic stem cells. *Exp. Hematol.*, **33**, 955–964 (2005).
 - 23) Laflamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK, Reinecke H, Xu C, Hassanipour M, Police S, O'Sullivan C, Collins L, Chen Y, Minami E, Gill EA, Ueno S, Yuan C, Gold J, Murry CE. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat. Biotechnol.*, **25**, 1015–1024 (2007).
 - 24) Jones CM, Dale L, Hogan BL, Wright CV, Smith JC. Bone morphogenetic protein-4 (BMP-4) acts during gastrula stages to cause ventralization of *Xenopus* embryos. *Development*, **122**, 1545–1554 (1996).
 - 25) Yatskievych TA, Ladd AN, Antin PB. Induction of cardiac myogenesis in avian pregastrula epiblast. The role of the hypoblast and activin. *Development*, **124**, 2561–2570 (1997).
 - 26) Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell*, **127**, 469–480 (2006).
 - 27) Naito AT, Shiojima I, Akazawa H, Hidaka K, Morisaki T, Kikuchi A, Komuro I. Developmental stage-specific biphasic roles of Wnt/beta-catenin signaling in cardiomyogenesis and hematopoiesis. *Proc. Natl. Acad. Sci. U.S.A.*, **103**, 19812–19817 (2006).
 - 28) Paige SL, Osugi T, Afanasiev OK, Pabon L, Reinecke H, Murry CE. Endogenous Wnt/beta-catenin signaling is required for car-

- diac differentiation in human embryonic stem cells. *PLoS ONE*, **5**, e11134 (2010).
- 29) Yang L, Soonpaa MH, Adler ED, Roepke TK, Kattman SJ, Kennedy M, Henckaerts E, Bonham K, Abbott GW, Linden RM, Field LJ, Keller GM. Human cardiovascular progenitor cells develop from a KDR⁺ embryonic-stem-cell-derived population. *Nature*, **453**, 524–528 (2008).
 - 30) Ueno S, Weidinger G, Osugi T, Kohn AD, Golob JL, Pabon L, Reinecke H, Moon RT, Murry CE. Biphasic role for Wnt/beta-catenin signaling in cardiac specification in zebrafish and embryonic stem cells. *Proc. Natl. Acad. Sci. U.S.A.*, **104**, 9685–9690 (2007).
 - 31) Caspi O, Huber I, Kehat I, Habib M, Arbel G, Gepstein A, Yankelson L, Aronson D, Beyar R, Gepstein L. Transplantation of human embryonic stem cell-derived cardiomyocytes improves myocardial performance in infarcted rat hearts. *J. Am. Coll. Cardiol.*, **50**, 1884–1893 (2007).
 - 32) Shiba Y, Fernandes S, Zhu WZ, Filice D, Muskheli V, Kim J, Palpant NJ, Gantz J, Moyes KW, Reinecke H, Van Biber B, Dardas T, Mignone JL, Izawa A, Hanna R, Viswanathan M, Gold JD, Kotlikoff MI, Sarvazyan N, Kay MW, Murry CE, Laflamme MA. Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. *Nature*, **489**, 322–325 (2012).
 - 33) Chong JJ, Yang X, Don CW, Minami E, Liu YW, Weyers JJ, Mahoney WM, Van Biber B, Cook SM, Palpant NJ, Gantz JA, Fugate JA, Muskheli V, Gough GM, Vogel KW, Astley CA, Hotchkiss CE, Baldessari A, Pabon L, Reinecke H, Gill EA, Nelson V, Kiem HP, Laflamme MA, Murry CE. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. *Nature*, **510**, 273–277 (2014).
 - 34) Shiba Y, Gomibuchi T, Seto T, Wada Y, Ichimura H, Tanaka Y, Ogasawara T, Okada K, Shiba N, Sakamoto K, Ido D, Shiina T, Ohkura M, Nakai J, Uno N, Kazuki Y, Oshimura M, Minami I, Ikeda U. Allogeneic transplantation of iPS cell-derived cardiomyocytes regenerates primate hearts. *Nature*, **538**, 388–391 (2016).
 - 35) Okano T, Yamada N, Sakai H, Sakurai Y. A novel recovery system for cultured cells using plasma-treated polystyrene dishes grafted with poly(*N*-isopropylacrylamide). *J. Biomed. Mater. Res.*, **27**, 1243–1251 (1993).
 - 36) Sekine H, Shimizu T, Dobashi I, Matsuura K, Hagiwara N, Takahashi M, Kobayashi E, Yamato M, Okano T. Cardiac cell sheet transplantation improves damaged heart function via superior cell survival in comparison with dissociated cell injection. *Tissue Eng. Part A*, **17**, 2973–2980 (2011).
 - 37) Kawamura M, Miyagawa S, Miki K, Saito A, Fukushima S, Higuchi T, Kawamura T, Kuratani T, Daimon T, Shimizu T, Okano T, Sawa Y. Feasibility, safety, and therapeutic efficacy of human induced pluripotent stem cell-derived cardiomyocyte sheets in a porcine ischemic cardiomyopathy model. *Circulation*, **126** (Suppl. 1), S29–S37 (2012).
 - 38) Masumoto H, Ikuno T, Takeda M, Fukushima H, Marui A, Katayama S, Shimizu T, Ikeda T, Okano T, Sakata R, Yamashita JK. Human iPS cell-engineered cardiac tissue sheets with cardiomyocytes and vascular cells for cardiac regeneration. *Sci. Rep.*, **4**, 6716 (2014).
 - 39) Weinberger F, Breckwoldt K, Pecha S, Kelly A, Geertz B, Starbatty J, Yorgan T, Cheng KH, Lessmann K, Stolen T, Scherrer-Crosbie M, Smith G, Reichenspurner H, Hansen A, Eschenhagen T. Cardiac repair in guinea pigs with human engineered heart tissue from induced pluripotent stem cells. *Sci. Transl. Med.*, **8**, 363ra148 (2016).
 - 40) 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*, **10**, e0131446 (2015).
 - 41) Strauer BE, Brehm M, Zeus T, Kosterling M, Hernandez A, Sorg RV, Kogler G, Wernet P. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation*, **106**, 1913–1918 (2002).
 - 42) Assmus B, Honold J, Schachinger V, Britten MB, Fischer-Rasokat U, Lehmann R, Teupe C, Pistorius K, Martin H, Abolmaali ND, Tonn T, Dimmeler S, Zeiher AM. Transcatheter transplantation of progenitor cells after myocardial infarction. *N. Engl. J. Med.*, **355**, 1222–1232 (2006).
 - 43) Schächinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Suselbeck T, Assmus B, Tonn T, Dimmeler S, Zeiher AM; REPAIR-AMI Investigators. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N. Engl. J. Med.*, **355**, 1210–1221 (2006).
 - 44) Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Ege-land T, Endresen K, Ilebakk A, Mangschau A, Fjeld JG, Smith HJ, Taraldsrud E, Grogard HK, Bjørnerheim R, Brekke M, Müller C, Hopp E, Ragnarsson A, Brinchmann JE, Forfang K. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *N. Engl. J. Med.*, **355**, 1199–1209 (2006).
 - 45) Chen S, Liu Z, Tian N, Zhang J, Yei F, Duan B, Zhu Z, Lin S, Kwan TW. Intracoronary transplantation of autologous bone marrow mesenchymal stem cells for ischemic cardiomyopathy due to isolated chronic occluded left anterior descending artery. *J. Invasive Cardiol.*, **18**, 552–556 (2006).
 - 46) Shiba Y, Filice D, Fernandes S, Minami E, Dupras SK, Biber BV, Trinh P, Hirota Y, Gold JD, Viswanathan M, Laflamme MA. Electrical integration of human embryonic stem cell-derived cardiomyocytes in a guinea pig chronic infarct model. *J. Cardiovasc. Pharmacol. Ther.*, **19**, 368–381 (2014).
 - 47) Menasché P, Vanneaux V, Hagege A, Bel A, Cholley B, Cacciapuoti I, Parouchev A, Benhamouda N, Tachdjian G, Tosca L, Trouvin JH, Fabreguettes JR, Bellamy V, Guillemain R, Suberbielle Boissel C, Tartour E, Desnos M, Larghero J. Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment. First clinical case report. *Eur. Heart J.*, **36**, 2011–2017 (2015).
 - 48) Suzuki K, Murtuza B, Beauchamp JR, Brand NJ, Barton PJ, Varela-Carver A, Fukushima S, Coppen SR, Partridge TA, Yacoub MH. Role of interleukin-1 β in acute inflammation and graft death after cell transplantation to the heart. *Circulation*, **110** (Suppl. 1), II-219–II-224 (2004).
 - 49) Frangogiannis NG. The inflammatory response in myocardial injury, repair, and remodeling. *Nat. Rev. Cardiol.*, **11**, 255–265 (2014).
 - 50) Murtuza B, Suzuki K, Bou-Gharios G, Beauchamp JR, Smolenski RT, Partridge TA, Yacoub MH. Transplantation of skeletal myoblasts secreting an IL-1 inhibitor modulates adverse remodeling in infarcted murine myocardium. *Proc. Natl. Acad. Sci. U.S.A.*, **101**, 4216–4221 (2004).
 - 51) Cardone MH, Salvesen GS, Widmann C, Johnson G, Frisch SM. The regulation of anoikis. MEKK-1 activation requires cleavage by caspases. *Cell*, **90**, 315–323 (1997).
 - 52) Reinecke H, Zhang M, Bartosek T, Murry CE. Survival, integration, and differentiation of cardiomyocyte grafts: a study in normal and injured rat hearts. *Circulation*, **100**, 193–202 (1999).
 - 53) Yoon SJ, Fang YH, Lim CH, Kim BS, Son HS, Park Y, Sun K. Regeneration of ischemic heart using hyaluronic acid-based injectable hydrogel. *J. Biomed. Mater. Res. B Appl. Biomater.*, **91B**, 163–171 (2009).
 - 54) Smith RR, Marban E, Marban L. Enhancing retention and efficacy of cardiomyocyte-derived cells administered after myocardial infarction using a hyaluronan-gelatin hydrogel. *Biomater.*, **3**, e24490 (2013).
 - 55) Ogasawara T, Okano S, Ichimura H, Kadota S, Tanaka Y, Minami I, Uesugi M, Wada Y, Saito N, Okada K, Kuwahara K, Shiba Y. Impact of extracellular matrix on engraftment and maturation of pluripotent stem cell-derived cardiomyocytes in a rat myocardial infarct model. *Sci. Rep.*, **7**, 8630 (2017).
 - 56) Matsushita S, Forrester JS, Li C, Sato M, Li Z, Guo X, Guan J,

- Amano A. Administration of cells with thermosensitive hydrogel enhances the functional recovery in ischemic rat heart. *J. Tissue Eng.*, **7**, 2041731416646676 (2016).
- 57) Frisco-Cabanas HL, Watanabe M, Okumura N, Kusamori K, Take-moto N, Takaya J, Sato S, Yamazoe S, Takakura Y, Kinoshita S, Nishikawa M, Koizumi N, Uesugi M. Synthetic molecules that protect cells from anoikis and their use in cell transplantation. *Angew. Chem. Int. Ed.*, **53**, 11208–11213 (2014).
 - 58) Yamazoe S, Shimogawa H, Sato S, Esko JD, Uesugi M. A dumb-bell-shaped small molecule that promotes cell adhesion and growth. *Chem. Biol.*, **16**, 773–782 (2009).
 - 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.*, **33**, 907–921 (2001).
 - 60) Kirby RJ, Divlianska DB, Whig K, Bryan N, Morfa CJ, Koo A, Nguyen KH, Maloney P, Peddibhotla S, Sessions EH, Hersherberger PM, Smith LH, Malany S. Discovery of novel small-molecule inducers of heme oxygenase-1 that protect human iPSC-derived cardiomyocytes from oxidative stress. *J. Pharmacol. Exp. Ther.*, **364**, 87–96 (2018).
 - 61) Melo LG, Agrawal R, Zhang L, Rezvani M, Mangi AA, Ehsan A, Griesse DP, Dell'Acqua G, Mann MJ, Oyama J, Yet SF, Layne MD, Perrella MA, Dzau VJ. Gene therapy strategy for long-term myocardial protection using adeno-associated virus-mediated delivery of heme oxygenase gene. *Circulation*, **105**, 602–607 (2002).
 - 62) Su CY, Chong KY, Chen J, Ryter S, Khardori R, Lai CC. A physiologically relevant hyperthermia selectively activates constitutive hsp70 in H9c2 cardiac myoblasts and confers oxidative protection. *J. Mol. Cell. Cardiol.*, **31**, 845–855 (1999).
 - 63) Yamashita N, Hoshida S, Nishida M, Igarashi J, Taniguchi N, Tada M, Kuzuya T, Hori M. Heat shock-induced manganese superoxide dismutase enhances the tolerance of cardiac myocytes to hypoxia-reoxygenation injury. *J. Mol. Cell. Cardiol.*, **29**, 1805–1813 (1997).
 - 64) Chinen J, Buckley RH. Transplantation immunology. Solid organ and bone marrow. *J. Allergy Clin. Immunol.*, **125** (Suppl. 2), S324–S335 (2010).
 - 65) Nakatsuji N, Nakajima F, Tokunaga K. HLA-haplotype banking and iPSC cells. *Nat. Biotechnol.*, **26**, 739–740 (2008).
 - 66) Nussbaum J, Minami E, Laflamme MA, Virag JA, Ware CB, Masino A, Muskheli V, Pabon L, Reinecke H, Murry CE. Transplantation of undifferentiated murine embryonic stem cells in the heart. Teratoma formation and immune response. *FASEB J.*, **21**, 1345–1357 (2007).
 - 67) Ahmed RP, Ashraf M, Buccini S, Shujia J, Haider H. Cardiac tumorigenic potential of induced pluripotent stem cells in an immunocompetent host with myocardial infarction. *Regen. Med.*, **6**, 171–178 (2011).
 - 68) Willems AR, Tijssen JG, van Capelle FJ, Kingma JH, Hauer RN, Vermeulen FE, Brugada P, van Hoogenhuyze DC, Janse MJ; The Dutch Ventricular Tachycardia Study Group of the Interuniversity Cardiology Institute of The Netherlands. Determinants of prognosis in symptomatic ventricular tachycardia or ventricular fibrillation late after myocardial infarction. *J. Am. Coll. Cardiol.*, **16**, 521–530 (1990).
 - 69) Roell W, Lewalter T, Sasse P, Tallini YN, Choi BR, Breitbach M, Doran R, Becher UM, Hwang SM, Bostani T, von Maltzahn J, Hofmann A, Reining S, Eiberger B, Gabris B, Pfeifer A, Welz A, Willecke K, Salama G, Schrickel JW, Kotlikoff MI, Fleischmann BK. Engraftment of connexin 43-expressing cells prevents post-infarct arrhythmia. *Nature*, **450**, 819–824 (2007).
 - 70) Liao SY, Liu Y, Siu CW, Zhang Y, Lai WH, Au KW, Lee YK, Chan YC, Yip PM, Wu EX, Wu Y, Lau CP, Li RA, Tse HF. Proarrhythmic risk of embryonic stem cell-derived cardiomyocyte transplantation in infarcted myocardium. *Heart Rhythm*, **7**, 1852–1859 (2010).
 - 71) Zhang YM, Hartzell C, Narlow M, Dudley SC Jr. Stem cell-derived cardiomyocytes demonstrate arrhythmic potential. *Circulation*, **106**, 1294–1299 (2002).
 - 72) Zhang J, Wilson GF, Soerens AG, Koonce CH, Yu J, Palecek SP, Thomson JA, Kamp TJ. Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ. Res.*, **104**, e30–e41 (2009).
 - 73) Lieu DK, Liu J, Siu CW, McNeerney GP, Tse HF, Abu-Khalil A, Huser T, Li RA. Absence of transverse tubules contributes to non-uniform Ca(2+) wavefronts in mouse and human embryonic stem cell-derived cardiomyocytes. *Stem Cells Dev.*, **18**, 1493–1500 (2009).
 - 74) Yang X, Rodriguez M, Pabon L, Fischer KA, Reinecke H, Regnier M, Sniadecki NJ, Ruohola-Baker H, Murry CE. Tri-iodo-L-thyronine promotes the maturation of human cardiomyocytes derived from induced pluripotent stem cells. *J. Mol. Cell. Cardiol.*, **72**, 296–304 (2014).
 - 75) Bedada FB, Chan SS, Metzger SK, Zhang L, Zhang J, Garry DJ, Kamp TJ, Kyba M, Metzger JM. Acquisition of a quantitative, stoichiometrically conserved ratiometric marker of maturation status in stem cell-derived cardiac myocytes. *Stem Cell Rep.*, **3**, 594–605 (2014).
 - 76) 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 Rep.*, **8**, 278–289 (2017).
 - 77) Schwartz SD, Hubschman JP, Heilwell G, Franco-Cardenas V, Pan CK, Ostrick RM, Mickunas E, Gay R, Klimanskaya I, Lanza R. Embryonic stem cell trials for macular degeneration. A preliminary report. *Lancet*, **379**, 713–720 (2012).