

## Review Article



# Cardiac Regeneration with Human Pluripotent Stem Cell-Derived Cardiomyocytes

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## ABSTRACT

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), which are collectively called pluripotent stem cells (PSCs), have emerged as a promising source for regenerative medicine. Particularly, human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) have shown robust potential for regenerating injured heart. Over the past two decades, protocols to differentiate hPSCs into CMs at high efficiency have been developed, opening the door for clinical application. Studies further demonstrated therapeutic effects of hPSC-CMs in small and large animal models and the underlying mechanisms of cardiac repair. However, gaps remain in explanations of the therapeutic effects of engrafted hPSC-CMs. In addition, bioengineering technologies improved survival and therapeutic effects of hPSC-CMs in vivo. While most of the original concerns associated with the use of hPSCs have been addressed, several issues remain to be resolved such as immaturity of transplanted cells, lack of electrical integration leading to arrhythmogenic risk, and tumorigenicity. Cell therapy with hPSC-CMs has shown great potential for biological therapy of injured heart; however, more studies are needed to ensure the therapeutic effects, underlying mechanisms, and safety, before this technology can be applied clinically.

**Keywords:** Pluripotent stem cells; Cardiomyocytes; Cell- and tissue-based therapy; Regeneration; Biomaterials

## INTRODUCTION

Cardiovascular Disease is the most common cause of deaths globally, accounting for more than 17 million deaths every year and accounting for 31% of all global deaths.<sup>1)</sup> Among them, ischemic heart disease including myocardial infarction (MI) causes 44% of deaths in the US.<sup>2)</sup> MI is associated with the death of myocardial tissue to a certain extent. Despite significant success in the treatment of acute MI by conventional pharmacological therapies, percutaneous coronary intervention, or coronary artery bypass graft, more than 15–30% patients still progress to heart failure (HF) with continuous loss and contractile dysfunction of cardiomyocytes (CMs) over the years.<sup>3,4)</sup> For end stage HF, heart transplantation is currently the only definitive treatment; however, it is limited by lack of donors, potential graft rejections, and various side effects resulting from immunosuppression.<sup>5)</sup>

# Conflict of Interest

The authors have no financial conflicts of interest.

# Author Contributions

Conceptualization: Yoon YS; Funding acquisition: Yoon YS, Park M; Investigation: Park M; Methodology: Park M; Supervision: Yoon YS; Validation: Yoon YS; Writing - original draft: Park M; Writing - review & editing: Yoon YS.

The adult human heart has minimal regenerative capacity with a CM renewal rate less than 1% per year.<sup>67)</sup> Thus, the ideal approach to heart regeneration after ischemic cardiac injuries is to provide target cardiac cells such as CMs by cell therapy for replacing the lost tissues. Earlier studies showed that various adult stem or progenitor cells are effective for cardiac repair in animal models. However, clinical trials with bone marrow-derived cells,<sup>8-16)</sup> mesenchymal stem cells,<sup>17)18)</sup> and cardiac progenitor cells<sup>19)</sup> have shown inconsistent results while showing their safety and feasibility.<sup>20)</sup> Moreover, unlike the original premise of stem cell therapy for direct cell or tissue generation, the therapeutic mechanisms of adult stem cells were found to be humoral or paracrine effects,<sup>21-25)</sup> including exosome-derived effects on preexisting cardiac tissue.<sup>26)</sup>

Recently, human pluripotent stem cells (PSCs), which refer to human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have emerged as promising cell sources for cardiac regeneration owing to their genuine property to differentiate into target cells such as CMs and endothelial cells (ECs). In this review, we will discuss the current status on the use of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) for cardiac regeneration. We will cover progress in the methods for differentiating hPSCs into CMs, the regenerative or therapeutic effects of differentiated hPSC-CMs on animal models of myocardial injury, bioengineering technologies to improve survival and therapeutic effects of hPSC-CMs, and the potential hurdles for clinical therapy with hPSC-CMs.

## DEVELOPMENT OF HUMAN PLURIPOTENT STEM CELLS

ESCs are derived from early embryos and have two distinct properties: a capacity for self-renewal and a capacity to differentiate into virtually any cell types, called pluripotency. After the first development of mouse ESCs in 1981,<sup>27)</sup> Thomson et al.<sup>28)</sup> successfully established human embryonic stem cells (hESCs) from the inner cell mass of human blastocysts in 1998. hESCs were then shown to be differentiated into various cell types including CMs and ECs and effective therapeutically.<sup>29)</sup> However, even in the form of differentiated cells, hESCs are not free of risks when being used therapeutically. They can form teratoma when undifferentiated cells are inadvertently included.<sup>30)</sup> Immune rejection due to immunological incompatibility between the cell and the recipient is another concern. Ethical concerns about destroying human embryos for generating hESCs are still unresolved.

Subsequently, Yamanaka and his colleagues<sup>31)32)</sup> made efforts to reprogram somatic cells into ESC-like cells, and finally succeeded in generating iPSCs using four transcription factors, *OCT4*, *SOX2*, *KLF4*, and *c-MYC*. The features of human induced pluripotent stem cells (hiPSCs) are almost identical to hESCs in the capacity for self-renewal and differentiation into multiple cell types. This reprogramming leads to the global reversion of the somatic epigenome into an ESC-like state.<sup>33)</sup> Genome-wide analysis indicated that iPSCs are very similar to ESCs.<sup>34)</sup> This development of hiPSCs made possible the use of patient-specific iPSCs for therapy, potentially eliminating the concern of immune rejections and ethical controversy associated with hESCs. While the use of genome-integrating viruses, such as retroviruses or lentiviruses in earlier studies limited its clinical applicability due to its potential for insertional mutation and tumor formation, successful generation of hiPSCs with non-genetic methods including episomal plasmid vectors<sup>35)36)</sup> adenovirus,<sup>37)</sup> Sendai virus,<sup>38)</sup> and modified mRNAs<sup>39)</sup> resolved this issue.

## DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO CARDIOMYOCYTES IN VITRO

To be used for cardiac regeneration, CMs must be generated from hPSCs. Since hPSCs are pluripotent, the cells should undergo differentiation into CMs. With clinical utility in mind, various approaches have been developed to meet the following requirements: 1) high yield or enrichment of CMs, 2) use of xenogeneic element-free media and defined components in differentiation protocols, and 3) scalability. Two basic approaches have been widely used for differentiating hPSCs to CMs: an embryoid body (EB)-mediated three-dimensional (3D) culture<sup>40)</sup> and a two-dimensional (2D) monolayer culture on extracellular matrix (ECM) proteins or feeders.<sup>41)</sup>

The EB-based differentiation initially involves suspending hPSC colonies by reversing the culture plates to form spherical aggregates, called EBs.<sup>40)</sup> These EBs contain differentiated cell types from all three germ layers, and once EBs are plated onto a feeder layer or ECM, spontaneously contracting areas develop in 5–15% of the EBs,<sup>40)42)</sup> usually after 10 days. These contracting EBs contain differentiated hESC-CMs, which exhibit spontaneous electrical activity with intracellular calcium transients and express cardiac markers such as MYH6 and -7, TNNT1, TNNT2, MYL-2A, MYL-2V, NPPA, ACTN, NKX2-5, and GATA4.<sup>40)</sup> However, due to the variability between different serum lots and the poorly defined factors in serum, this protocol is hard to reproduce, and the efficiency is low (<1% from hESCs). To improve the differentiation efficiency, various measures were added to this protocol. Xu and colleagues<sup>43)</sup> added a Percoll gradient centrifugation step to obtain enriched (up to 70%) populations of hESC-CMs. The suspension culture of EB and forced aggregation methods produced a high number of functional CMs.<sup>44)</sup> However, these methods are technically complex, time consuming, and associated with line-to-line variation. This pitfall has led to the development of monolayer-based 2D-culture method.

The 2D system allows uniform exposure of cells to exogenous soluble factors in the media and yields higher and more consistent differentiation efficiency. An early approach for 2D culture or directed differentiation methods used mouse visceral endoderm-like cells (END-2) as a feeder layer which produces Activin-A and BMPs, among other factors, resulting in an increased contracting area in more solid aggregates.<sup>45)</sup> This protocol, while relatively inefficient, has been shown to generate mostly ventricular-like CMs.<sup>45)</sup> This technique was improved using a small molecule inhibitor of p38MAP kinase, which almost doubled the yield of hESC-CMs by enhancing induction of mesoderm.<sup>46)</sup> More sophisticated methods were developed later by modifying signaling pathways that regulate formation and patterning of heart from cardiac mesoderm such as NODAL/Activin-A, WNT/ $\beta$ -Catenin, and BMP4.<sup>47-50)</sup> Signals mediated through WNT/ $\beta$ -catenin and TGF- $\beta$  family members including Activin and BMPs promote differentiation of ESCs into mesoderm.<sup>51-53)</sup> Once mesoderm is induced, however, WNT/ $\beta$ -catenin signaling inhibits cardiac differentiation, suggesting biphasic roles of WNT signaling in cardiomyogenesis.<sup>53)54)</sup> Laflamme et al.<sup>29)</sup> reported that high density culture of hPSCs with addition of Activin-A followed by 4 days of BMP4 generated contracting cells at day 12 with a purity of approximately 30% CMs. A combined density-gradient centrifugation enriched the yield to 80–90%. Combining Matrigel and growth factors (Activin-A, BMP4, FGF2), termed ‘matrix sandwich’ method,<sup>55)</sup> increased the purity (up to 98%) and yield (up to 11 CMs/input hPSC),<sup>55)</sup> suggesting the importance of ECM for hPSC-CM differentiation. However, Matrigel may limit the clinical utility of the protocol because it potentially includes xenogeneic pathogens and has a significant lot-to-lot variation.

Another method using Matrigel without growth factors generated a high yield of hPSC-CMs ~90%; however, it required manual selection of beating cells.<sup>56)</sup> More recently, Burrige and colleagues reported a chemically defined method using solely small molecules on synthetic matrices, producing CMs at >85% purity.<sup>57)</sup>

Despite remarkable improvement in the methods generating hPSC-CMs, these in vitro protocols can still produce heterogeneous cell populations including undifferentiated hPSCs or non-CMs, which may elicit off-target outcomes. Therefore, enrichment for CMs became a critical issue for clinical utility. The methods for enriching hPSC-CMs are diverse, and are covered in another review of ours<sup>58)</sup>: 1) density centrifugation,<sup>43)</sup> 2) genetic modification,<sup>59)</sup> 3) surface protein-based enrichment,<sup>60)</sup> 4) MITO tracker-based enrichment,<sup>61)</sup> 5) Lactate-based enrichment,<sup>62)</sup> 6) mRNA-based molecular beacon,<sup>63)</sup> 7) microRNA-based enrichment,<sup>64)</sup> and 8) microfluidic systems.<sup>65)</sup> At present, antibody-based and lactate-based methods are widely used. Another attempt was made to generate chamber-specific CMs by modulating only signaling pathways without using purification methods. Keller and colleagues demonstrated a transgene-independent method for the generation of sinoatrial node-like pacemaker cell (SANLPC) from hPSCs (85%) by stage-specific manipulation of developmental signaling pathways,<sup>66)</sup> while the beating rate paced by SANLPC were much faster (~137 bpm) than human resting heart rate. The same group also succeeded in generating atrial and ventricular CMs from distinct mesoderm populations.<sup>67)</sup> The availability of subtypes of CMs (atrial-like, ventricular-like,<sup>67)</sup> or sinoatrial nodal-like cells<sup>66)</sup> will expand the applicability of hPSC-CMs from cell therapy to the modeling of specific cardiac disorders and drug discovery.

## THERAPEUTIC EFFECTS OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES ON MYOCARDIAL INJURY

The feasibility and efficacy of hPSC-CMs for cardiac regeneration after myocardial injury were tested in animal models including mouse,<sup>68)</sup> rat<sup>29)69)</sup> and pig.<sup>70)71)</sup> Earlier animal studies have shown that hPSC-CMs can partially remuscularize infarcted areas and attenuate adverse cardiac remodeling and HF, while electrical coupling with host hearts were not shown.<sup>29)69)</sup> In a guinea pig model, transplanted hESC-CMs showed electric coupling to native myocardium, suggesting that the low heart rate of guinea pig can better model the electrical activities of grafted hPSC-CMs.<sup>72)</sup> On the other hands, when delivered into chronic MI in rats and guinea pigs,<sup>73)74)</sup> hPSC-CMs did not have a significant beneficial effect on cardiac function, and had limited electromechanical integration. Although rodent models of MI provided information of functional benefit,<sup>29)69)72)75)</sup> preclinical studies with non-human primate models needed to be attempted to better evaluate the therapeutic effects and the safety of hPSC-derived CMs in cardiac injury.<sup>76)77)</sup> The first clinical-scale study was done with macaque monkeys. Here, intra-myocardial injection of hESC-CMs remuscularized infarcted hearts two weeks after ischemia reperfusion (I/R) injury. A substantial number of grafted hPSC-CMs survived over three months after I/R, where they formed electromechanical junctions with the host heart and beat in synchrony without forming teratoma or having off-target effects.<sup>78)</sup> However, the authors did not conclude potential benefits of hESC-CMs in human cardiac disease, mainly due to the small numbers of study animals (n=7) and the unrealistic number of transplanted cells (~10 billion CMs for human application, extrapolating from 2–3 billion CMs for monkey).<sup>79)</sup> Although arrhythmias were not observed in small animal studies, in monkeys

a transient period of ventricular arrhythmias was seen.<sup>78)</sup> More recently, Murry's group<sup>80)</sup> reported that the transplantation of ~750 million cryopreserved hESC-CMs improved left ventricular ejection fraction (LV-EF) (10.6% at 1 week and additional 12.4% at 3 months) with restoration of contractile function after ischemic injury in macaque monkeys. This study demonstrated that remuscularization of the infarcted non-human primate heart with hESC-CMs exerted robust and durable improvement in cardiac function without detectable graft-induced arrhythmias. Zhu et al.<sup>81)</sup> also examined the safety and efficacy of hPSC-derived cardiovascular progenitor cells (hPSC-CVPCs) on MI (cell injection 30 minutes after induction of MI) in cynomolgus monkeys, but could not find remuscularization of infarcted heart or any transplanted cells at 20 weeks after transplantation. The discrepancy might have come from the difference in experimental details such as the disease model (I/R vs. MI), timing of cell delivery (2 weeks vs. 30 minutes after ischemic injury), and the type and the dose of transplanted cells (~1 billion hESC-CMs vs. 10 million hPSC-CVPCs).<sup>81)</sup>

## BIOENGINEERING APPROACHES TO ENHANCE THERAPEUTIC EFFECTS OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

One of the main problems in cell therapy is low survival of the transplanted cells.<sup>82-84)</sup> While a few studies demonstrated robust remuscularization, many studies showed poor survival of hPSC-CMs in ischemic hearts when cells were transplanted alone, with most of them disappearing within a month.<sup>85)</sup> As such, to improve poor retention and survival of transplanted cells, diverse biomaterials and tissue engineering technologies have been attempted over the past decade.<sup>79)96)97)</sup> Two major strategies are cell delivery with an injectable biomaterial and cell delivery in a form of engineered tissue patches.<sup>86)</sup>

### Injection of cells encapsulated with biomaterials

Injectable biomaterials or hydrogels are most frequently used to deliver cells to injury sites and were the first strategy explored to improve engrafted cell retention and survival in heart.<sup>87)</sup> In general, such injectable biomaterials were shown to enhance cell survival and promote tissue regeneration.<sup>88)89)</sup> While hydrogels are commonly composed of synthetic polymers, native ECM components can also form hydrogels.<sup>90)91)</sup> Naturally-derived biomaterials used for encapsulation of stem cells for cardiac regeneration include biodegradable polypeptide (silk fibroin from worms and insects), polysaccharide-based materials (chitosan from crustacean shells,<sup>92)93)</sup> alginate from brown algae,<sup>94)</sup> agarose from red algae, hyaluronic acid, collagen), and fibrin derived from blood plasma.<sup>87)</sup> Synthetic biomaterials include peptide amphiphiles incorporating cell adhesive ligands (injectable nanomatrix gel)<sup>89)</sup> and other polymer-based materials. It is noted that biomaterial structures dictate degradation and controlled release of therapeutics into ischemic myocardium.<sup>86)</sup> Natural biomaterials have better biocompatibility and degradability; however, they are not controllable. Synthetic biomaterials are modular but elicit more inflammatory reactions. There is accumulated evidence that hPSC-CM encapsulation with proper injectable biomaterials improves engrafted cell survival and promotes cardiac repair.<sup>95)</sup> In selecting biomaterials, bioactivity<sup>96)97)</sup> and mechanical properties need to be considered as well. ECM hydrogels are biocompatible and have been used in preclinical applications for MI.<sup>98)</sup> Another study showed that functional output and contractility of engrafted hPSC-CMs might be dependent on substrate mechanical stiffness.<sup>99)</sup>

### Bioengineered artificial cardiac tissue or patch

To avoid engrafted cell death in the harsh tissue environment, especially the infarcted scar area, a tissue patch was generated by incorporating biomaterials and hPSC-CMs and was implanted onto the surface of the infarcted area. Current tissue patch approaches include hydrogel-based engineered heart tissue,<sup>100-103</sup> scaffold-free cell sheets,<sup>71,104,105</sup> and 3D-printed cardiac tissue with a complex ECM structure.<sup>106,107</sup> The commonly used scaffold materials for hydrogel-based engineered heart tissue with hPSC-CMs are ECMs such as collagen I,<sup>100</sup> Matrigel,<sup>108</sup> and fibrin.<sup>101,102</sup> Other natural biomaterials such as hyaluronic acid-based hydrogel were also attempted for engineering PSC-CMs.<sup>109</sup> When these ECM-based heart tissue containing hPSC-CMs were transplanted onto ischemic heart models, inconsistent results were observed in improvement of cell survival and LV function depending on the injury model and the types of transplanted cells. One study transplanted physically integrated cardiac tissue sheets containing hiPSCs (hiPSC-CTs) onto infarcted rat hearts and found a significant improvement of cardiac function with >40% of cells engrafted at 4 weeks after transplantation.<sup>105</sup> On the other hand, the transplantation of collagen-based heart tissue containing hESC-CMs in nude rats one month following I/R injury showed no significant improvement of LV-EF compared to a patch containing nonviable hESC-CMs,<sup>100</sup> raising a question about the necessity for hPSC-CMs in the patch. Other studies with fibrin-based heart tissue combining multiple cells such as hiPSC-CMs + hiPSC-ECs (5:2 ratio)<sup>101</sup> or hiPSC-CMs + hiPSC-ECs + hiPSC-SMCs (4:2:2 ratio)<sup>102</sup> improved contractile function and engrafted cell survivals (~10%) one month after surgery. Recently, Bursac and colleagues demonstrated that a patch consisting of hydrogel containing fibrinogen and Matrigel and a combination of cells (~86% of hiPSC-CMs, ~14% of fibroblasts + SMCs) exhibited electrical and mechanical function similar to those of the adult myocardium.<sup>103</sup> When transplanted, however, the cells within the patch remained in the patch, not migrating into the heart. Scaffold-free cell sheets with hiPSC-CMs were created to improve cardiac contractility in a porcine model of ischemic cardiomyopathy<sup>71</sup> and a rat model<sup>110</sup> of acute MI. However, the beneficial effects of the cardiac cell sheet were only transient due to the lack of oxygen and nutrient supply into the transplanted sheets. To solve this problem, another study combined hiPSC-CMs sheets with an omental flap.<sup>104</sup> Three months after transplantation of this sheet with an omental flap into infarcted porcine heart, cardiac contractile function was significantly improved. A 3D printing technology has attracted attention for cardiac tissue engineering. A multiphoton-excited 3D printing technique produced ECM-based scaffolds containing hiPSC-CMs, hiPSC-ECs, and iPSC-SMCs (2:1:1 ratio), termed cardiac muscle patch (hCMP).<sup>107</sup> When transplanted onto the heart, hCMPs increased contraction speed and calcium handling in a mouse MI model. Although these tissue-engineered patches have multiple benefits for cell delivery and retention in host ischemic myocardium, this approach still has limitations such as non-migration of cells into the host heart, the need for open-chest surgery, arrhythmic risks associated with large graft size, and biodegradability of included biomaterials. This bioengineered cardiac tissue approach would better fit into the treatment of chronic MI or HF in which more mechanical support is needed.

## CHALLENGES TO THE USE OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES FOR CLINICAL THERAPY

However, there are still other hurdles along the way to clinical application of hPSC-derived CM for cardiac regeneration.

### Cellular heterogeneity

Current differentiation protocols produce a mixture of non-CMs and different subtypes of CMs, such as ventricular, atrial, and nodal CMs.<sup>111)112)</sup> As mentioned above, various sorting or enriching methods were developed to generate a pure population of CMs. Although ventricular CMs are predominant in the culture,<sup>113)</sup> this subtype diversity and contamination of non-CMs may induce graft-related arrhythmias and aberrant tissue formation. While antibody-based and metabolism-based methods are most widely used<sup>114)</sup> at present, the efficiency is variable according to the cell line and cells become weak when metabolically selected.

### Immature phenotype

hPSC-derived CMs show immature characteristics with less-organized sarcomeric structures and calcium handling properties.<sup>115-117)</sup> Studies reported that hPSC-derived CMs are closer to fetal CMs than adult CMs in terms of maturity. hPSC-CMs have round morphology (vs. rod-shaped),<sup>118)</sup> use glucose metabolism (vs. fatty acid),<sup>115)</sup> and do not have T-tubules.<sup>119)120)</sup> Several methods to enhance maturation of hPSC-CMs in vitro have been developed. Prolonged in vitro culture (80–120 days) induced a phenotype of adult CMs including increase of cell size and contractile properties.<sup>113)121)</sup> Three-dimensional culture with electrical stimulation through biowire generated a mature type of CMs.<sup>117)</sup> Overexpression of the let-7 family of microRNA in hESC-CMs enhanced cell size, sarcomere length, and contractile force.<sup>122)</sup> microRNA-499 also promoted ventricular specification of hESCs and microRNA-1 served to facilitate electrophysiological maturation.<sup>123)</sup> ECM was also reported to mature hPSC-CMs, including decellularized adult cardiac ECMs.<sup>124)125)</sup> In addition, in vivo maturation of hPSC-CMs were demonstrated in engrafted hPSC-CMs in rat hearts with faster maturation in the infarcted adult rat hearts compared to neonatal rat hearts.<sup>126)</sup> However, hPSC-CMs transplanted into pig hearts did not show sufficient maturation after long-term follow-up. Thus far, no one can say what the optimal stage of hPSC-CMs is for cardiac transplantation. It is generally accepted that a certain intermediary maturation state may be ideal,<sup>127)</sup> since adult CMs do not survive transplantation.<sup>128)</sup>

### Arrhythmogenicity

To function appropriately, the engrafted hPSC-derived CMs at the ischemic myocardium should integrate electrically with host myocardium to beat in synchrony and avoid arrhythmias. Multiple factors including functional immaturity of transplanted hPSC-CMs and lack of electrical integration can induce arrhythmia.<sup>79)129)</sup> In small animal models, arrhythmia was not frequently reported presumably due to the rapid heart rate of rodents.<sup>74)</sup> However, in large animal models, hPSC-CMs transplantation induced a transient period of ventricular arrhythmias.<sup>78)130)</sup> Since the transplanted hPSC-CMs are immature and have various CM subtypes, the large grafts have higher risk of life-threatening arrhythmia. However, more recent studies demonstrated that even in swine<sup>102)</sup> or non-human primate models of MI,<sup>78)80)</sup> transplantation of hPSC-derived CMs can only induce non-sustained and less frequent ventricular arrhythmias. Further studies are needed to address the risk of arrhythmia associated with hPSC-CM transplantation.

### Tumorigenicity

Undifferentiated PSCs can form teratoma when injected into the heart of immunocompromised animals.<sup>30)</sup> It is reported that iPSCs have a higher survival rate than adult stem cells, and hiPSCs develop teratoma more efficiently and faster than hESC.<sup>131)</sup> However, it is still controversial whether hPSC-derived differentiated cells can form teratoma. Even if the risk of teratoma formation is one of the major safety concerns for cell therapy with

hPSC-derived cells, to date, there is no evidence for teratoma formation after transplantation of hPSC-derived CM<sup>80)</sup> or ECs.<sup>132)</sup> However, the potential for teratoma formation by hPSC-derived cell grafts should be taken seriously<sup>133)</sup> because 1) animal xenograft models may not accurately predict the fate of grafted cells in humans, 2) it is hard to guarantee the complete absence of undifferentiated cells in large scale cell production for the patient, and 3) current assays may not correctly assess tumorigenic potential. While there are efforts to develop surrogate markers for cell transformation and to determine the threshold level of residual stem cells which pose a risk for teratoma development, ultimately long-term follow-up studies in animals and pilot clinical trials can answer this question.

## CONCLUSION

At present, hPSC-CMs are the only realistic option for meaningful remuscularization of injured heart. Scientists made substantial progress in the development of generating hPSC-CMs, even approaching clinical quality and scale, toward the understanding of the biology of hPSC-CMs and their behaviors in vivo following transplantation, and the engineering methods to enhance the cell survival and therapeutic effects. However, many concerns remain to be resolved before their translation into clinical use. While development of human iPSCs avoids the ethical concerns for the use of human ESCs, potential side effects associated with the pluripotency of stem cells and the appropriate stage of hPSC-CMs for clinical use must be addressed before hPSC-CMs become a clinical reality.

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