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Human Cardiac Tissue Engineering: From Pluripotent Stem Cells to Heart Repair

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Abstract

Engineered cardiac tissues hold great promise for use in drug and toxicology screening, *in vitro* studies of human physiology and disease, and as transplantable tissue grafts for myocardial repair. In this review, we discuss recent progress in cell-based therapy and functional tissue engineering using pluripotent stem cell-derived cardiomyocytes and we describe methods for delivery of cells into the injured heart. While significant hurdles remain, notable advances have been made in the methods to derive large numbers of pure human cardiomyocytes, mature their phenotype, and produce and implant functional cardiac tissues, bringing the field a step closer to widespread *in vitro* and *in vivo* applications.

INTRODUCTION

Heart disease is a significant cause of death worldwide. Over 60% of cardiac-related deaths result from occlusion of coronary arteries [1] leading to adverse ventricular remodeling, arrhythmias, and heart failure. Current clinical interventions for ischemic heart disease involve revascularization via angioplasty or bypass grafting, which can prevent further ischemia but do not replace irreversibly lost cardiomyocytes (CMs). Cell and tissue transplantation therapies have thus emerged as potential approaches for repairing the injured heart via addition of new functional CMs [2].

Among a number of candidate cell sources for cardiac repair, human pluripotent (embryonic or induced) stem cells (hPSCs) have emerged as an attractive option due to their unique ability to generate unlimited numbers of functional cardiomyocytes [3]. In this review, we will discuss recent advances in the use of hPSC-derived CMs (hPSC-CMs) for cardiac repair, including studies of hPSC-CM differentiation and maturation, fabrication of human cardiac tissue equivalents, and delivery of cells and therapeutic molecules for on-site cardiac repair. Selected literature reports utilizing non-human CMs, which present novel findings and/or methods that could be applied to hPSC-CMs, are also described.

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DIFFERENTIATION AND PURIFICATION OF hPSC-CMs

Numerous protocols for differentiating hPSCs into CMs have been reported, with most following the common paradigm of sequential activation and inhibition of the Wnt signaling pathway [4]. Efficient CM differentiation has been achieved in both adherent and suspension cultures via treatment with growth factors Activin A and BMP4 [5], or small molecules (CHIR99021, IWP2) [6]. These protocols can produce up to 95% CMs with yields of up to 15 CMs per input hPSC without additional purification, though high levels of variability have been reported among different cell lines. Thus, several modifications have been made to improve reproducibility and efficiency of original protocols, including the use of a “Matrigel sandwich” prior to treatment with Activin A and BMP4 to promote epithelial-mesenchymal transition (EMT) of hPSCs during early differentiation and increase CM purity and yield [7]. Similar outcomes have been recently reported using small molecule-based differentiation in chemically defined medium, which could greatly improve differentiation consistency and promote efficient generation of clinical grade cells [8].

Although reliable protocols for obtaining relatively pure populations of hPSC-CMs have been developed, additional optimization and standardization is required to increase purity and enable robust, large-scale generation of hPSC-CMs for clinical applications. Additionally, the safety and success of any hPSC-based therapy is critically dependent on the elimination of proliferative and potentially teratoma-forming cells from the differentiated CM population. CM purification from a mixed population of hPSC-derived cells can be accomplished by non-genetic methods using fluorescence-activated or magnetic-activated cell sorting (FACS or MACS, respectively) based on cardiac-specific expression of surface markers SIRPA or VCAM1 [9] or elevated mitochondrial content measured by the TMRM dye [10]. These sorting techniques, however, are limited in throughput and can negatively affect cell health. Thus, a technique for CM purification was developed based on the ability of CMs, but not non-CMs, to metabolize lactate instead of glucose as an alternative energy source [11]. Culturing hPSC derivatives in glucose-free, lactate-enriched media results in a CM purity of 95-99% and can easily be scaled up to large culture vessels. These methods may potentially be combined with other general methods for eliminating residual undifferentiated hPSCs, such as treatment with small molecules [12], to ensure safety in eventual clinical use.

While the highest possible purity of hPSC-CMs has been the goal of many differentiation protocols, it is important to note that the presence of other cardiac-relevant cells such as fibroblasts, endothelial cells, and smooth muscle cells may be beneficial for both maturation of CMs and engineering of highly functional cardiac tissues. Liao et al. [13] found that a small percentage of cardiac fibroblasts (3-12 %) were required for murine PSC-CMs to elongate and form a functional syncytium when encapsulated in a three-dimensional (3D) fibrin hydrogel matrix. More recently, similar results have been obtained for engineered tissues formed from non-dissociated aggregates of differentiated murine or human PSC-CMs (termed cardiac bodies), where a fibroblast percentage of 8-17% resulted in optimal functional properties [14]. Others have reported that the presence of 25% non-cardiomyocytes was most advantageous for hPSC-CM differentiation compared to 0%, 50%, and 75% cells [15]. In all cases, the ability of supporting non-myocytes to

mechanically remodel the surrounding extracellular matrix (ECM) as well as exert paracrine effects on CMs appears critical for the formation of functional cardiac tissue.

MATURATION OF hPSC-CMs

It has been well-established that compared to CMs in the adult heart, CMs derived from pluripotent stem cells are structurally and functionally immature, displaying properties characteristic of the developing fetal heart [16,17]. Among the changes that occur during the maturation of CMs from fetal to adult phenotype are a marked increase in cell size, change in cell shape from round to rod-like, polarization of electrical and mechanical junctions, formation of T-tubules, increased contractile force generation, and increased upstroke velocity of the action potential [16].

Culturing hPSC-CMs for long periods of time (up to 120 days) can increase cell size and advance CM maturation [18]. Accelerating the maturation process to faster than physiological times is critical given that human CMs in the native heart do not reach maturity until several years post-birth [19]. Recently, thyroid hormone T3 was found to increase cell size and improve electrophysiological and contractile function of hPSC-CMs grown in monolayers [20]. Interestingly, T3 was also proven essential for proliferation of CMs in the maturing postnatal rodent heart *in vivo* [21], despite the observed anti-proliferative effect on human PSC-CMs *in vitro* [20] and on fetal sheep CMs *in vivo* [22]. Future studies are needed to elucidate the effects of T3 on immature CMs, with the understanding that results could be species-dependent.

Three-dimensional culture can further improve structural, genetic, and electromechanical maturation of hPSC-CMs compared to age-matched 2D cultures, as shown in our previous study [23]. The use of a similar system for the 3D culture of primary neonatal rat ventricular myocytes resulted in the formation of T-tubules and co-localization of L-type calcium channels with ryanodine receptors [24], providing the first evidence of advanced maturation state in non-adult cardiomyocyte culture. Since the optimal amount of mechanical load is known to dynamically regulate T-tubule structure in the native heart [25], culturing hPSC-CMs in a 3D environment with appropriate temporal changes in cellular loading and ECM stiffness could be an important component of their maturation process *in vitro*.

TISSUE ENGINEERING

In addition to maturation of individual hPSC-CMs, an important consideration for engineering of functional cardiac tissues is assembly of CMs into a multicellular network with the ability to replicate the tissue-level electrical and contractile function of native myocardium (Figure 1). This requires the dense, ordered arrangement of aligned CMs within an ECM scaffold as well as the formation of functional gap junctions to provide intercellular current flow and adherens junctions to provide intercellular force transmission. Several techniques have been used to build functional cardiac tissues from hPSC-CMs, including scaffold-free cell sheets[26], fibrin-based [23,27,28] or collagen-based [14,15,29-31] hydrogels, and porous gelatin scaffolds [32]. Despite recent progress, both electrical (Table 1) and mechanical functionality of engineered hPSC-CM tissues are vastly inferior to those of the adult myocardium [16]. Since CMs in the native heart are constantly subjected to

electromechanical activation through rapid conduction of action potentials followed by calcium-induced contractions, recent efforts have been focused on utilizing electrical or mechanical stimulation to enhance functional cardiogenesis of hPSC-CMs.

In a recent study, chronic electrical stimulation of hPSC-CM tissues resulted in increased velocity of action potential conduction, CM size, and amplitude of calcium release [29], while other studies have shown positive effects on the amplitude of generated contractile force [27]. Potential beneficial effects of cyclic stretch, intended to mimic ventricular filling, have also been investigated. When applied to hPSC-CMs seeded on gelatin sponges, cyclic stretch upregulated the expression of gap junctions, ion channels, and sarcomeric proteins and enhanced calcium handling [32]. As an alternative to cyclic stretch, progressive lengthening during culture (growth stretch) has been applied to 3D hPSC-CM tissues to mimic heart growth during development [14]. The growth stretch improved CM alignment and sarcomeric structure, which was not observed for cyclic stretch in the same tissue model. Despite this progress, electromechanical properties of hPSC-CMs and engineered tissues remain to be significantly improved to allow for use in reliable *in vitro* assays [33] and safe and efficient therapies. As researchers continue to study methods for improving PSC-CM differentiation *in vitro*, culture systems that allow continuous and non-invasive monitoring of functional changes in cardiac cells and tissues [31,34,35] may enable active tuning of culture conditions to accelerate the maturation process.

CARDIAC REPAIR

Following cardiac injury, PSC-CMs can be delivered to the heart by implanting an engineered tissue patch or injecting cell suspension (Figure 2). Implantation of tissue patches requires open-heart surgery, although patches engineered using cell sheet technology can be attached to epicardial surface without use of sutures or surgical glues [36]. Cell injections, on the other hand, can be performed percutaneously including a catheter delivery from the endocardial side, which does not require surgery [37]. Intracoronary catheter injections are used to further minimize the invasiveness of procedure by infusion of cells into the coronary blood vessels without puncturing the heart [38]. Regardless of the injection protocol, a significant drawback of delivering cell suspension in the heart is the poor survival and retention of injected cells [39]. The use of *in situ* polymerizable hydrogel solutions for cell delivery could enhance the retention of injected cells without a need for traditional open-heart surgery, although injectable hydrogels were not as effective as epicardial tissue patches in promoting cell retention [40]. In addition to hPSC-CM transplantation, delivery of drugs and proteins, either alone or combined with transplanted cells, will be critical for successfully remuscularization and revascularization of the injured heart. For example, a “pro-survival cocktail” containing anti-apoptotic peptides, immunosuppressant small molecules, and growth factors (IGF-1) has been co-delivered with hPSC-CMs to enhance their engraftment in the heart [41]. For longer-term effects following cell engraftment, controlled drug release from a degradable scaffold [42] combined with hPSC-CM therapy could provide further benefits to cell survival, blood vessel ingrowth, and functional integration with host tissue.

Of the numerous studies employing PSC-CMs for treatment of cardiac injury in animal models (Table 2), the two recent reports by Shiba et al [43] and Chong et al [44] provided the best evidence to date that grafted hPSC-CMs can functionally integrate with the host heart to replace lost myocardial tissue. In both studies, hPSC-CMs were engineered to express the fluorescent calcium indicator GCaMP3, which allows donor cell-specific visualization of calcium transients following graft implantation. GCaMP3-labeled hPSC-CMs were injected into cryoinjured guinea pig hearts [43] or infarcted macaque hearts [44], and the authors confirmed that grafted cells exhibited calcium transients indicative of functional CMs. Furthermore, in some [43] or all [44] of the animals, GCaMP3 transients were synchronized with the ECG of the heart, indicating electrical coupling between graft and host. While the small sample size, excessive numbers of implanted cells, and arrhythmogenicity are notable concerns for immediate translation of these studies to clinics [45], this work represents unequivocal proof that grafted hPSCCMs can survive, remain functional, and electrically couple with the native heart. A variety of other studies have shown positive effects of PSC-CM implantation on compromised cardiac function (Table 2); still, relative roles of direct functional integration vs. paracrine effects of the implanted PSC-CMs remain to be defined.

FUTURE CHALLENGES AND DIRECTIONS

Recent advances in differentiation and purification of hPSC-CMs and proof-of-principle studies showing hPSC-CM survival and integration in large animal hearts hold great promise for future use of these cells in clinical practice. Nonetheless, significant barriers exist in producing mature hPSC-CMs and engineering cardiac tissues with near-adult levels of electrical and contractile function. Even tissues constructed from neonatal rodent cells exhibit far inferior functional properties to adult rodent myocardium despite their advanced differentiation state (neonatal) compared to hPSC-CMs (fetal) and shorter cardiac maturation time in rodents (weeks [46,47]) than humans (years [19]). Considering that approximately half a billion adult CMs are lost in a human myocardial infarction [48] and that the adult CMs are ~50 times larger and orders of magnitude stronger-contracting than hPSC-CMs [16] [49], employing mature CMs, either injected or in a form a preformed 3D tissue patch, will be critical for successful functional repair of infarcted human hearts. While implanting a tissue patch could enhance cell survival and retention compared to cell injection [50], it remains to be shown if implanted human cardiac patches, similar to injected hPSC-CMs [43,44], can survive, remain functional, and electrically couple with host myocardium. Moreover, enhanced nutrient and oxygen delivery via functional vasculature and/or perfusion will be vital for the successful engineering of large-size, clinically relevant patches to enable efficient cardiac repair [51].

With these considerations in mind, continued efforts to improve the maturation of early-stage hPSCCMs and assemble 3D cardiac tissues with functional properties approaching those of the adult myocardium are of paramount importance. Due to their relative ease of preparation and low price, rodent primary or PSC-derived CMs remain valuable model systems for investigating techniques to mature CMs and engineer robust cardiac tissues. Relevant experiences with rodent cells can be applied to hPSC-CMs as more reproducible and economical protocols for human cell differentiation and purification are emerging.

Biophysical and biochemical cues that normally guide cardiac development *in vivo* [52] may be applied *in vitro*, however, specific culture regimes have to be verified for the induction of true physiological response rather than short-term compensatory or non-physiologic changes. It is important to note that most chronic electrical and mechanical stimulation regimes in rodent cells are applied at sub-physiological rates and, thus, directly extrapolating the results to human cells requires caution. On the other hand, better understanding of what governs the faster developmental clock in rodents vs. humans is likely to yield robust protocols to accelerate hPSC-CM maturation.

CONCLUSIONS

The great interest in human pluripotent stem cell-derived cardiomyocytes and engineered cardiac tissues for regenerative medicine applications, drug and toxicology screening, and studies of human physiology and disease, has led to fast progress in this multidisciplinary and dynamic field. The last few years have been focused on developing reproducible and lower-cost approaches for efficient differentiation of hPSC-CMs, improving their maturation state *in vitro*, and defining their predictive value as preclinical drug safety assays. Engineered cardiac tissues hold potential to advance maturation of cardiomyocytes to near adult-levels, but will require the ability to efficiently vascularize and integrate *in vivo* before they could move toward clinical use. Improved methods to co-deliver cells, biomaterials, and therapeutic molecules will pave the way for future progress. Successful safety and efficacy studies in large animals and increased cost-effectiveness are additional requirements to enable translation. With the present degree of enthusiasm and rapidly paced discoveries, we are optimistic that the dream of cardiac regenerative therapy will soon become a reality.

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Highlights

- Notable progress in cardiac differentiation of human pluripotent stem cells (hPSCs)
- Lack of robust methods to mature hPSC-derived cardiomyocytes (hPSC-CMs) *in vitro*
- Engineered tissues from hPSC-CMs may advance CM maturation to near-adult levels
- Multiple methods developed to deliver hPSC-CMs to injured myocardium
- hPSC-CMs shown to electrically couple with host CMs in small and large animals

Figure 1.

General workflow for engineering and testing functional hPSC-CM tissues. hPSCs are differentiated to hPSC-CMs via a multi-stage differentiation procedure utilizing growth factors, small molecules, or their combination and purified via surface marker or metabolic selection. To form 3D engineered cardiac tissues, purified hPSC-CMs are combined with different biomaterials, further matured by application of biophysical and biochemical stimuli, and tested for structural, functional and molecular properties. Therapeutic potential of hPSC-CM tissues is evaluated by their implantation onto an injured heart followed by *ex vivo* and *in vivo* assessments.

Figure 2. Methods for delivery of cells into an injured heart. (1) Intracoronary cell infusion from a catheter in the coronary vasculature. (2) Intramyocardial injection from an epicardially inserted needle. (3) Transendocardial injection from an endocardially inserted catheter needle. (4) Epicardial cell delivery in the form of a tissue patch.

Table 1Functional properties of *in vitro* engineered 3D hPSC-CM tissues

Scaffold for Tissue Engineering	Conduction Velocity (cm/s)	Action Potential (Ca ²⁺ transient) Duration (ms)	Maximum Capture Rate (Hz)	Contractile Force (mN)	Specific Force (mN/mm ²)	Reference
Collagen gel	4.9 cm/s	-	-	1.37	4.4	[14]
Fibrin gel	25	250-400	3.5	3.0	11.8	[23]
Collagen gel	15	120	4	-	-	[29]
Collagen gel	~15	~350	6	-	-	[15]
Decellularized mouse heart	~1	(~300)	4	0.18	-	[53]
Collagen gel	-	245	3	~1	0.66	[31]
Fibrin gel	-	-	-	0.083	~1.2-2.6	[27]
Fibrin gel	-	341-887	-	0.061	0.12	[28]
Collagen gel	-	-	-	~0.1	0.08	[30]
Gelatin sponge	-	(500)	-	-	-	[32]
Native Tissue						
Adult human ventricle	46.4	350-430	3.9	-	25-44	[54-58]

~ values estimated from figures.

Table 2

Effects of hPSC-CM grafting in animal cardiac injury models

Implantation Method	Animal Model	Injury Type	Treatment Time	Notable Outcomes	Reference
Cell sheet	Pig	MI	Chronic	<ul style="list-style-type: none"> • ↑ EF, vascularization • ↓ interstitial fibrosis 	[26]
Cell injection	Rat	MI	Sub-acute	<ul style="list-style-type: none"> • ↑ EF, FAS • ↓ Dilation 	[41]
Cell injection	Rat	MI	Sub-acute	<ul style="list-style-type: none"> • ↑ FAS • ↓ Dilation 	[59]
Cell injection	Mouse	MI	Acute	<ul style="list-style-type: none"> • ↑ EF at 4 weeks but not sustained at 12 weeks 	[60]
Cell injection	Rat	MI	Chronic	<ul style="list-style-type: none"> • No functional benefit of transplantation 	[61]
Cell injection	Guinea pig	Cryo-injury	Chronic Sub-acute	<ul style="list-style-type: none"> • Electrical coupling, graft-host synchronization • ↓ arrhythmias in sub-acute but not chronic injury 	[43,62]
Cell injection	Macaque	MI	Chronic	<ul style="list-style-type: none"> • Electrical coupling, graft-host synchronization • Non-fatal arrhythmias 	[44]
Cell Injection	Mouse	MI	Acute	<ul style="list-style-type: none"> • ↑ EF • ↓ Infarct area 	[63]
Gelatin scaffold	Rat	MI	Sub-acute	<ul style="list-style-type: none"> • ↑ Preservation of graft size after cyclic stretch <i>in vitro</i> 	[32]

Abbreviations: MI – myocardial infarction; EF – ejection fraction; FAS – fractional area shortening. Treatment times: Acute – on the day of injury; Sub-acute – less than 10 days post-injury; Chronic – more than 10 days post-injury;