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Human-Induced Pluripotent Stem Cell-Derived Engineered Cardiac Tissues

Takeichiro Nakane, Hidetoshi Masumoto and
Bradley B. Keller

Additional information is available at the end of the chapter

Abstract

By combining tissue engineering techniques with human-induced pluripotent stem cell (hiPSC) technology, human-derived engineered cardiac tissues (ECTs) have been developed using several cell lineage compositions and 3-dimensional geometries. Although hiPSC ECTs are relatively immature compared with native adult heart tissues, they have promising potential as a platform technology for drug-screening and disease modeling, and as grafts for hiPSC-based regenerative heart therapy. This chapter provides the focused overview of the current status of cardiac tissue engineering technology and its possible application.

Keywords: iPS cell, engineered cardiac tissue, tissue engineering, drug screening, disease modeling, cardiac regeneration

1. Introduction

With the progress of tissue engineering technology in the last decade, many kinds of engineered cardiac tissues (ECTs) have been developed and reported. These tissues possess striated myofibers which recapitulate unique contractile function of heart tissues. Takahashi, Yamanaka, and colleagues developed iPSCs from mice in 2006 and from human next year [1, 2]. Human iPS cells (hiPSCs) have the potential to differentiate into cardiomyocytes and other cardiac lineage cells. Recently, the efficiency of cardiac differentiation has rapidly improved, which makes it possible to robustly induce cardiac lineage cells [3–6]. By using cardiac cells derived from hiPSCs as a cell source for ECT generation, the potential of ECTs has expanded. The present chapter overviews the current status of ECT technology and its possible application.

2. Methods for generating engineered cardiac tissues

2.1. Cell sheet technology

Okano, Shimizu and colleagues have developed a culture surface grafted with temperature-responsive polymer, poly(N-isopropyl acrylamide) (PIPAAm) [7]. This system enables confluent cells to detach themselves from the surface while maintaining a sheet structure simply by reducing the culture temperature. The cell sheets are fabricated without the use of exogenous matrices. It has endogenous matrix layer at the bottom side, and it secures biological attachment to the recipient heart surface within 30 min [8]. This scaffold-free tissue is a thin sheet, and three-dimensional thicker structure can be obtained by stacking several sheets. However, stacking more than three layers (approximately 80 μm thickness) triggers cell death at the center due to hypoxia [9]. At our lab, we overcame this limit by stacking cell sheets with gelatin hydrogel microspheres (GHM) [10]. Five-layered cell sheets with GHM displayed better cell viability *in vitro* compared with the control cell sheet without GHM. Furthermore, cell sheet modification with GHM improved the retention of grafted cell sheets on the rat heart epicardial surface after myocardial infarction. We succeeded in generating over 1 mm thick constructs from 15 sheets using this technology.

2.2. Biomaterials

Cardiomyocytes and other cells are embedded in biomaterials, such as collagen I, matrigel, and/or fibrin in a casting mold. Exogenous gel matrix promotes self-assembly of cells leading to form a tissue structure. The first successful cardiac tissue fabrication by this method was reported in the year 1997 [11]. Fixed anchors in these molds generate static strain, which enhances the cell alignment and contractile function. The geometry of mold controls the final tissue architecture, and a variety of tissue shapes have been formulated, including linear [12–16], circular [17], and mesh structures (**Figure 1**) [18–20]. By applying engineering manufacturing methods, it may be possible to automate the process of generating small-sized ECTs, leading to the development of high throughput *in vitro* analysis systems [21, 22]. In fact, recently, 3D bio-printing technology has been applied and provided the possibility of creating more complicated structures reproducibly by printing both scaffold matrix and living cells [23].

2.3. Prefabricated matrix

Biomaterials provide a 'soft' environment for the cells to grow in. On the other hand, synthetic microporous ('spongy') scaffolds made from alginate, collagen, and gelatin or other stiffer materials, such as polystyrene, PLLA (Poly-L-Lactide Acid), PLGA (Poly(lactic-co-glycolic acid)) and PCL (Polycaprolactone), have also been tested [24, 25]. The advantage of this method is that these rigid scaffolds contribute to engineering any desired structure and size with mechanical stability. However, the maturation of various mesodermal cells is matrix stiffness dependent, and therefore both the biomaterial gel and the supporting mold may impact cell maturation and survival [26].
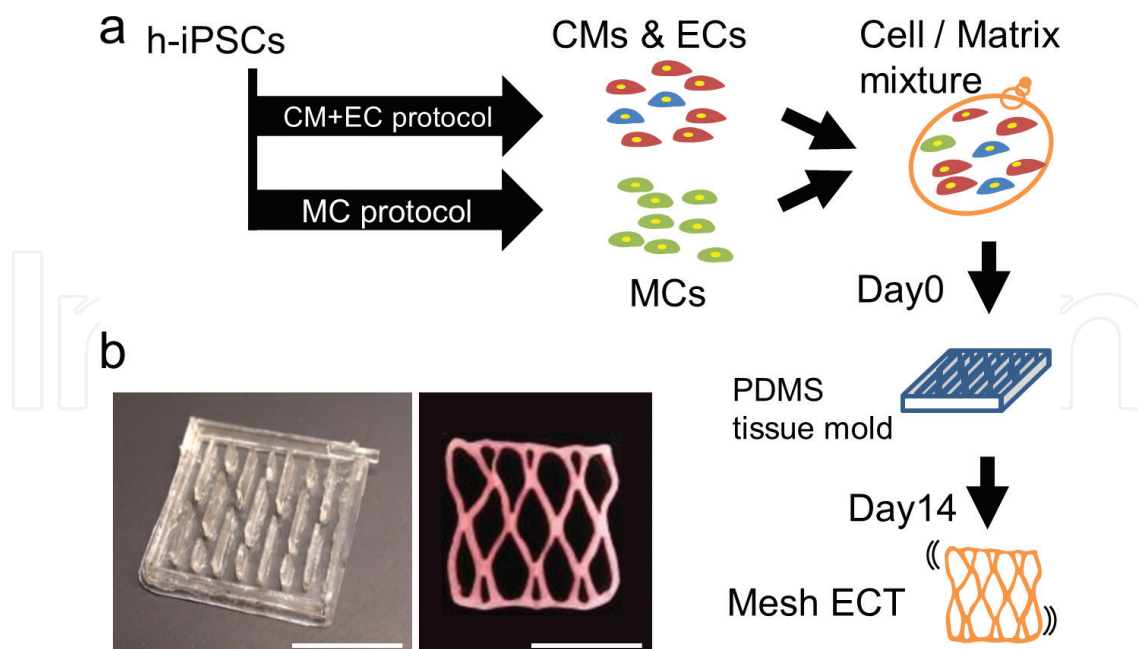
Figure 1. Generation of a mesh ECT by biomaterial-based technique. (a) Schematic diagram for a mesh ECT generation. Cardiomyocytes (CM), endothelial cells (EC), and mural cells (MC) are induced from human iPS cells (h-iPSCs) using two different protocols. Cells and collagen-based matrix were combined and poured into custom polydimethylsiloxane (PDMS) molds at day 0 and cultured for 14 days. (b) Representative image of a PDMS mold (left) and a mesh ECT (right). Scale bar: 10 mm. (Reproduced from Ref. [20] with permission).

2.4. Decellularized tissue

Decellurization of a whole heart is achieved by the treatment with sodium dodecyl sulfate (SDS) and Triton X-100 under Langendorf perfusion [27]. The decellularized tissue maintains most of the tissue contents and function of the extracellular matrix. Hence, decellularized tissue provides a native scaffold which can support repopulation by mesoderm lineage cells. These decellularized tissues may be advantageous in the generation of *in vitro* cardiac tissues [28, 29].

3. Maturation of the tissue

3.1. Cellular contents

A heart tissue is composed of multiple cell types including cardiomyocytes, fibroblasts, endothelial cells, and smooth muscle cells. In human hearts, cardiomyocytes account for 25-50% of total cells, occupying around 70% of the whole volume [30]. Many reports have demonstrated that non-myocytes support the maturation of cardiomyocytes and play a significant role in both myocardial structure and function [13, 16, 19].

We induced cardiomyocytes (CM), endothelial cells (EC), and vascular mural cells (MC) from hiPSCs, and generated ECTs from several formulations of cardiomyocytes and non-myocytes,

such as CM+EC, CM+MC, and CM+EC+MC [16]. According to in vitro force measurement, CM+EC+MC ECTs showed most advanced electrophysiological properties. Furthermore, histological analysis revealed CM+EC+MC possessed more unidirectionally aligned myofiber with mature sarcomeric structures (**Figure 2**).

The mechanism for the improvement of tissue maturation as the result of a more complex lineage mixture is still unknown. Evidence is emerging that cardiomyocytes and fibroblasts are electrically coupled, which may modulate electrophysiologic function [31]. Direct interaction of different cell types or paracrine effects can be considered. It is reported that extracellular matrix derived from cardiac fibroblast supported the proliferation *in vitro* and indicated the usefulness of the coculture [32].

3.2. Effects of extended culture duration on CM and ECT maturation and function

Cardiomyocytes derived from pluripotent stem cells mature early in culture but are arrested at the late embryonic stage under 2-dimensional (2D) culture condition [33]. Meanwhile, it is widely recognized that cardiomyocytes in a 3-dimensional (3D) cardiac tissue acquire a more mature phenotype than those in 2D culture [25, 34].

In our study, we prolonged culture duration of mesh ECTs from 14 to 28days [20]. Long-cultured constructs showed the tendency to augment the active contractile force along with the increase of beating frequency from 1.5Hz to 2.5Hz and maintained greater force compared to 14-day constructs. The shift from a negative to a neutral force-frequency relationship in 28-day constructs represents sustained functional maturation as well as more rapid force generation and relaxation cycle, and the ability to capture higher pacing frequency. Additional culture duration enhanced myofiber alignment along with the expression of several genes related to ion channels and gap junction (**Figure 3**).

3.3. Impact of culture condition on ECT maturation and function

In order to expand the dimension of ECTs, it is necessary to improve the distribution of oxygen and nutrient throughout tissues. Direct perfusion of culture medium reduces the gradients associated with diffusional mass transport between the construct surfaces and the inner phase and improves the microenvironmental conditions within ECTs. [35–37]. In addition to the increase of cellularity at the center of ECTs, perfusion system contributes to the maturation of cardiomyocytes and the tissue structure. It is reported that even the simple rocking dynamic culture yields engineered myocardium with near-adult functional output [38]. The system accelerates force generation and conduction velocity probably due to the activation of mTOR signaling.

For generating ECTs, especially from biomaterial-based technique, materials from other sources, such as collagen derived from rat or matrigel, have been widely used with xeno-serum containing culture medium. Tiburcy and colleagues modified their methods toward clinical application and developed a formulation for generating ECTs by using medical grade bovine collagen and maintaining them under serum-free defined condition [19]. This modification provided a proof-of-concept for a universally applicable technology for the engineering
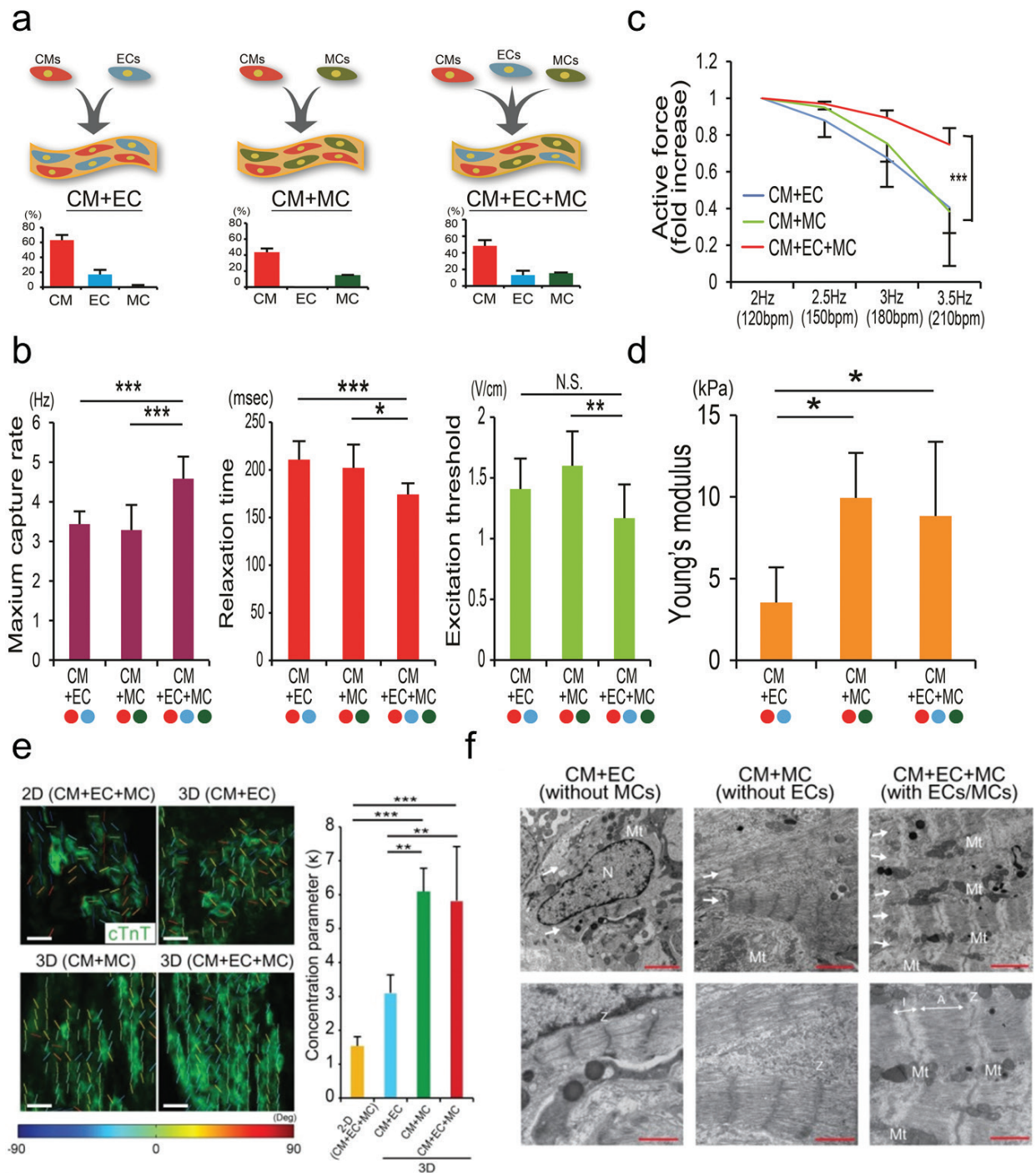
Figure 2. Electromechanical properties and structural maturation of hiPSC-ECTs. (a) Schematic diagrams for generating 3 types of ECTs containing cardiomyocytes (CM), endothelial cells (EC), and/or mural cells (MC) (upper) and the proportions of each cell type (lower) [n = 8 (CM+ EC), 7 (CM+ MC), and 12 (CM+ EC+ MC). (b–d) Results of contractile force measurements [n = 8 (CM+ EC), 7 (CM+ MC), and 12 (CM+ EC+ MC)]. (b) Maximum capture rate (left), relaxation time (center) and excitation threshold voltage (right) (c) Relationship between active force and pacing frequency (2Hz to 3.5Hz). (d) Young's modulus of ECTs. NS, not significant; *P < 0.05,**P < 0.01, ***P < 0.001. (e) Representative alignment analysis using cTnT-stained images after 2-dimensional (2D) and 3-dimensional (3D) culture for 3 types of ECTs (left). Calculated concentration parameter (κ) (right) [n =3 (2D) and 5 (each 3D)]. Larger values of κ represent greater alignment to a single direction. cTnT, cardiac troponin T; Deg, degree. (f) Representative transmission electron microscopic images for each type of ECT cultured for 4 weeks. Arrows indicate myofibers. N, nucleus; Mt, mitochondria; I, I-band; A, A-band; Z, Z-line. (Reproduced from Ref. [16] with permission).
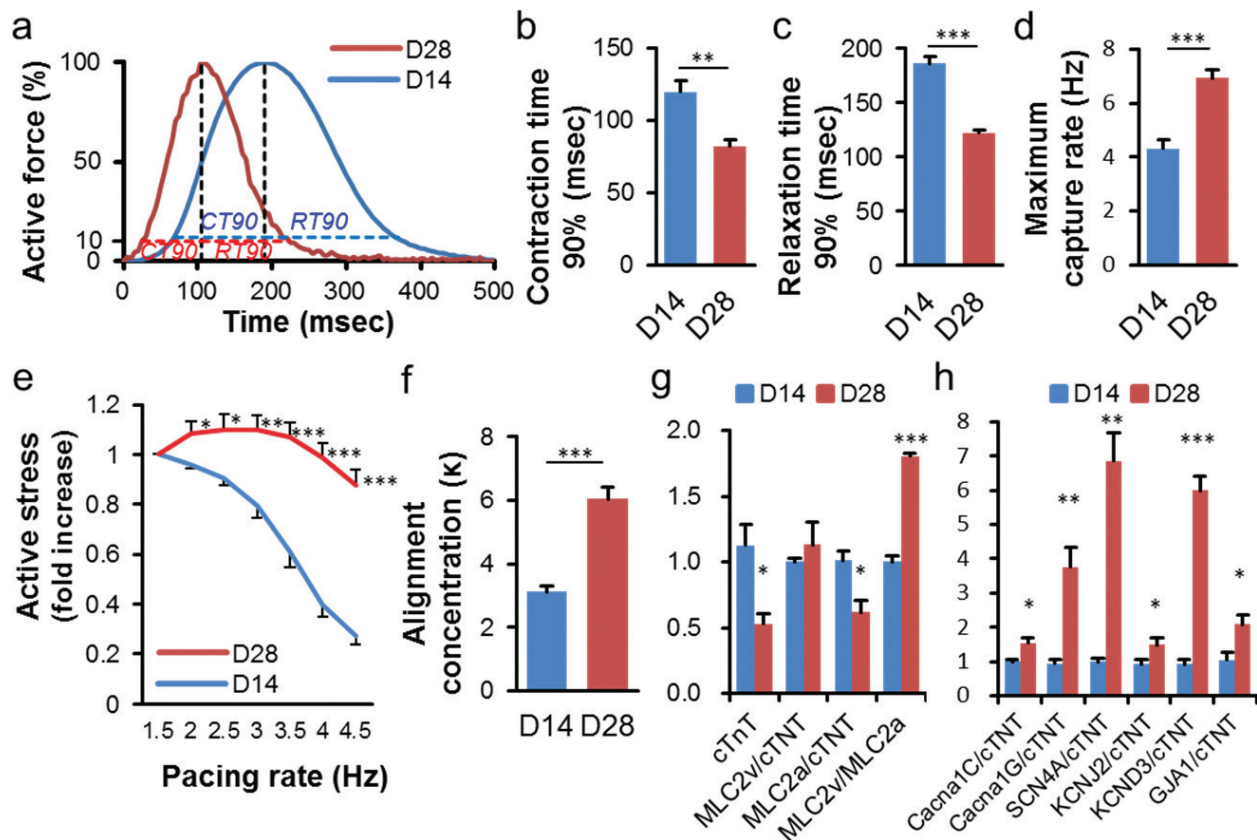
Figure 3. Effects of extended culture duration on mesh ECT maturation. (a) Averaged normalized active force-time curves for mesh ECT cultured for either 14 (blue, D14) or 28 (red, D28) (n = 6) days under 2 Hz electrical stimulation. Contraction time (CT) or relaxation time (RT) represents the time of 90% increase or decrease of force respectively. Comparison of (b) contraction time 90% (n = 6; ***P < 0.01), (c) relaxation time 90% (***P < 0.001) at 2 Hz pacing, (d) maximum capture rate (n = 6; ***P < 0.001), (e) force-frequency relationship (n = 6; *P < 0.05 to ***P < 0.001 vs. D14), and (f) cardiomyocyte alignment concentration (κ), [n = 4 (D14) and n = 3 (D28); ***P < 0.001]. (g), (h) Q-PCR analysis (n = 3; *P < 0.05 to ***P < 0.001). cTnT level was lower in D28, and other genes were normalized to the value of cTnT expression. (Reproduced from Ref. [20] with permission).

cardiac tissue. In addition, ECTs generated by this condition displayed advanced cardiomyocyte maturation compared to the conventional methods [19].

3.4. Role of mechanical loading on ECT maturation and function.

Several *in vitro* ECT studies revealed the impact of uniaxial mechanical loading on the alignment of cardiomyocytes and functional maturation. The simplest system for that is the two fixed anchors which generate static strain on the tissue between them. Furthermore, cyclic stress conditioning markedly increases cardiomyocyte hypertrophy and proliferation rates versus unconditioned constructs [13]. We demonstrated that ECTs displayed a broad spectrum of altered gene expression in response to cyclic stretch, reflecting a complex regulation of proliferation, differentiation, and architectural alignment of cardiomyocytes and non-cardiomyocytes within ECTs [39].

Electrical stimulation is considered to be another important cue for further maturation of tissues [40]. Nunes and colleagues subjected their engineered tissues, named biowires,

on electrical stimulation at up to 6 Hz [41]. Biowires subjected to electrical stimulation had markedly increased myofibril ultrastructural organization, elevated conduction velocity and improved both electrophysiological and Ca^{2+} handling properties compared to non-stimulated controls. These changes were in agreement with cardiomyocyte maturation and were dependent on the stimulation frequency.

Another group created a system which applied combined electromechanical stimulation mimicking isovolumic contraction and confirmed the improvement of functional properties over electrical or mechanical stimulation alone. In the report, the timing of the combined stimulation greatly affected the electrophysiological properties of ECTs [42].

4. Applications of ECTs

4.1. Drug screening

Cardiotoxicity is one of the main causes of drug withdrawal from the market [43, 44]. To evaluate the safety and effectiveness of drugs, several kinds of pre-clinical studies are performed using non-human models. However, these models occasionally show incorrect results, such as pseudo-negative, due to the difference of electrophysiology in other species [45]. By using hiPSC technology, effects to humans can be examined from the early stage [46]. Moreover, hiPSC-derived ECTs are expected to be more specific and sensitive platforms for drug screening [14].

We have developed a 3D cardiac tissue model which reproduces Torsade de Pointes (TdP) showing both a typical polymorphic extracellular field potential and meandering spiral wave re-entry upon treatment with IKr channel blockers [47]. It is generated from hiPSC-derived cardiomyocytes and mesenchymal cells using the cell sheet technology. The appearance of TdP-like waveforms was significantly higher in this 3D model compared with 2D monolayer conditions indicating that the multilayered 3D structure is an essential factor for this arrhythmia along with the coexistence of non-cardiomyocytes.

Many groups have developed various high throughput screening formats, such as strip format [48] and heart-on-chip [21, 49], and confirmed the similarity of hiPSC-derived engineered tissues with native heart tissues [14, 50–52]. Video optical recording system set up in a usual CO_2 incubator is also useful to continuously monitor contractile abilities of tissues [48].

Cardiomyocytes from hiPSCs are at the premature stage, and even ECTs generated to date remain immature compared to native adult myocardium. Hence, further cues for maturation as described above is necessary to imitate native tissues more precisely [51].

4.2. Disease modeling

iPS cells derived from a patient with a known genetic mutation for the disease can provide the disease model, which may offer a useful strategy for understanding the mechanism of the disease and exploring a new treatment modality.

Cardio-facio-cutaneous syndrome (CFCS) is one of the RASopathies, and cardiac abnormalities are the most common findings among CFCS, including hypertrophic cardiomyopathy (HCM) in approximately 40% of patients. Nearly 75% of patients with CFCS exhibit mutations in *BRAF*, which encodes a serine/threonine kinase and a direct effector of Ras. Cashman and colleagues created 3D human engineered cardiac tissue model of HCM using human cardiomyocytes yielded by directed differentiation of iPSCs established from a patient with CFCS carrying an activated BRAF mutation [53].

Chronic catecholamine overstimulation contributes to heart failure progression. Overstimulation of ECTs with norepinephrine provides a simulation of a human heart failure
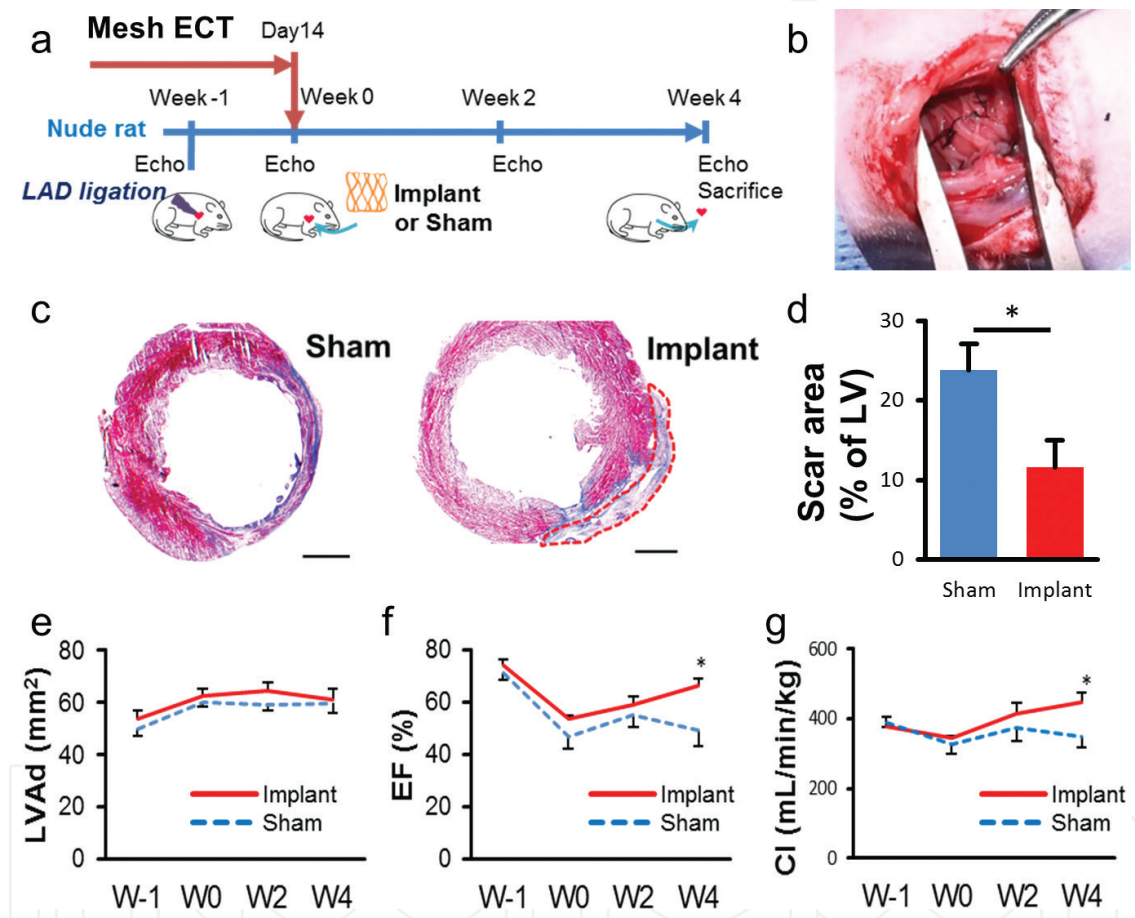

Figure 4. Therapeutic effects of hiPSC-mesh ECT implantation in a rat myocardial infarction model. (a) Schematic timeline of surgery. A mesh ECT matured *in vitro* for 14 days (or sham suture) is implanted in a nude rat (week 0) 1 week after the induction of myocardial infarction by ligating left anterior descending artery (LAD). Echocardiogram (Echo) is performed prior to LAD ligation at week-1 (W-1), prior to implantation at week 0 (W0), then week 2 (W2) and week 4 (W4). (b) Grafted mesh ECT on the heart surface covering infarction site. (c) Representative Masson's trichrome staining images of sham treated (left) and mesh ECT implanted (right) rat hearts at W4. Scale bar: 2 mm. Red dotted line indicates engrafted area. (d) Comparison of scar area (% of LV area) at W4 (n=5, *P<0.05 Implant versus Sham). (e-g) Results of echocardiogram [n=5 (Implant, red solid line) and 5 (Sham, blue dotted line)]. (e) Left ventricular end diastolic area (LVAd; mm^2), (f) ejection fraction, EF (%), and (g) cardiac index, CI (mL/min/kg) (*P<0.05 Implant versus Sham at W4). (Reproduced from Ref. [20] with permission).

phenotype [19]. Tissues responded to chronic catecholamine toxicity with contractile dysfunction, cardiomyocyte hypertrophy, cardiomyocyte death, and NT-proBNP release, which are classical hallmarks of heart failure. Notably, the pathological phenotype could be partially or fully prevented by β1- or α1-adrenoreceptor blockade, demonstrating the applicability of ECTs in the *in vitro* simulation of heart failure and its prevention by pharmacological means.

4.3. Transplantation therapy

HiPSCs are now one of the most promising cell sources for cardiac regenerative cell therapy [54–56]. There are major methods of cell delivery, including intracoronary or intramuscular injection of dispersed cells and epicardial transplantation of engineered tissues [57]. It is possible to deliver a large number of differentiated cells with organized architecture by ECT implantation. The grafted tissues survive and support the heart wall, which overcomes the problem of poor retention rate following cell injection [34].

A variety of studies has revealed the efficacy of ECT implantation for myocardial structural and functional recovery in injured hearts of several animal models [6, 10, 58, 59]. We implanted ECTs in an athymic nude rat myocardial infarction model. ECTs with vascular cells displayed the invasion of vasculature from the host heart to the tissue and their perfusion. Survived ECTs replaced the ventricular wall in the injured area and prevented the scar formation after myocardial infarction and improved cardiac function (**Figure 4**). ECTs survived during 4-week follow-up period [16, 20]. However, further work is required to identify the underlying mechanism for the functional recovery. Meanwhile, the first transplantation of cardiac progenitor patch derived from human embryonic stem cells in a severe heart failure patient was performed in France, offering an encouraging result [60].

5. Conclusion

In this chapter, we have reviewed several aspects of current cardiac tissue engineering technologies and presented the possible applications of these tissues for *in vitro* drug toxicity testing, human disease modeling, and paradigms for myocardial recovery with muscle replacement following injury. This rapidly evolving new field is now incorporating manufacturing process to expand the scalability and reduce the cost of generating these novel engineered *in vitro* myocardial tissues.

Conflict of interest

None.

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